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CYTOLOGICAL ASSAY OF C-MITOTIC AND PROPHASE POISON ACTIONS*

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With 10 Figures in the Text

(Eingegangen am 26. Oktober 1957)

In a previous paper (BOWEN and WILSON, 1954) the cytological effects induced by several antibiotics were compared with those produced by colchicine. Two general conclusions were drawn; (1) that the antibiotics used (actidione, streptomycin, and chloromycetin) could be classed as prophase poisons as defined by D'AMATO (1948) and (2) that the prophase poison and c-mitotic effects were the result of very different types of reactions. The present paper represents an extension of this work aimed primarily at providing better quantitative descriptions of the two reactions.

Actidione was used as the prophase poison and colchicine as the c-mitotic agent. Both drugs were obtained from the same source as previously, but were from different batches. The testing technique was essentially that described by BOWEN and WILSON (1954) and subsequently referred to as the *Pisum* test. Essentially this test consists of treating standard size (2.5—3.5 cm) primary roots of pea seedlings under standard conditions and examining their meristems for cytological changes relative to dose-time changes. In so far as facilities were available every effort was made to control conditions during both germination and treatment. All points shown on "reaction" curves are the means of not less than three root tips per point.

The colchicine reaction

From work reported by other authors, previous studies in this laboratory and pilot runs during the course of the present study, the following general conclusions appear inevitable:

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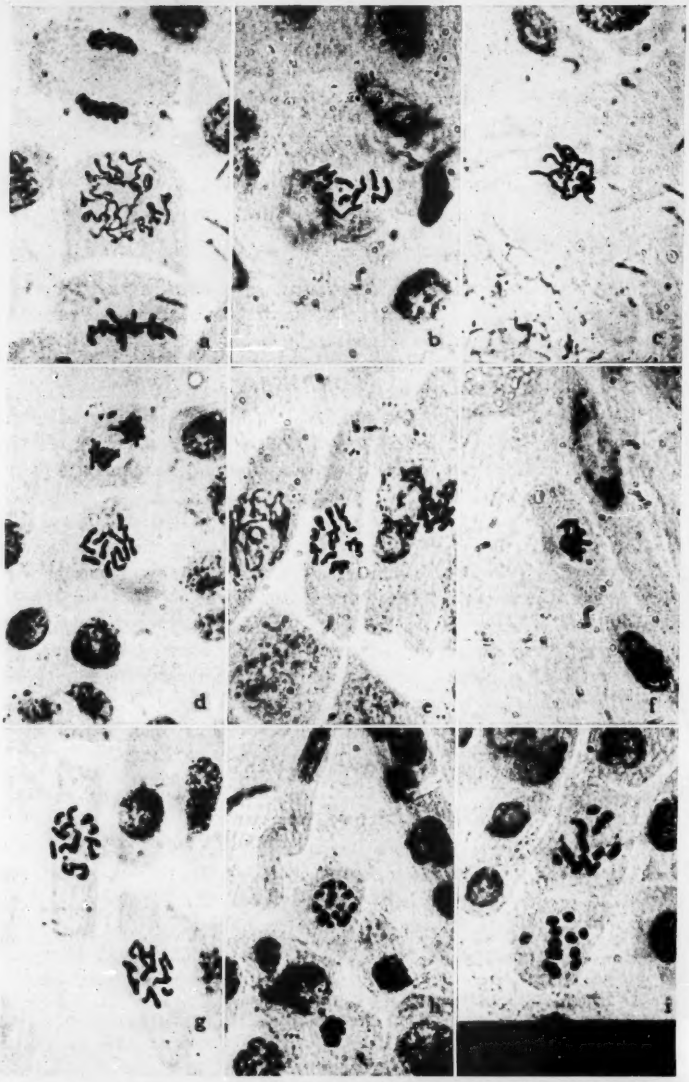

Fig. 1a—i. a—c normal mitosis in *Pisum*; d—f colchicine configurations; g—i actidione configurations

1. Two characteristic mitotic configurations are found in treated material. One is the "scattered metaphase" (Fig. 1d and e) or classic c-metaphase of LEVAN (1938) and others and is especially associated with low dose, high dose for short times and with recovery from high dose (BOWEN and WILSON, 1954; HYPPIO, TSOU and WILSON, 1955). The second is a clumped configuration (Fig. 1f) which appears to represent a stalled prometaphase especially conspicuous in peas and in the ascites tumor (LEVAN, 1954). This configuration is associated with high doses of short duration and moderate doses for comparatively long times and also appears to be the configuration most likely to lead to polyploidy.

2. The degree of effect increases with both increase in dose and in duration of treatment.

3. No effect on the progression of events prior to late prophase can be demonstrated.

Table 1. *Concentration conversion table*

Molar concentration × 10^{-6}		Parts per Million	Percent
Actidione	Colchicine		
3550.0	2506.0	1000	0.1000
1775.0	1253.0	500	0.0500
355.0	250.6	100	0.0100
265.5	188.3	75	0.0075
177.0	125.5	50	0.0050
141.6	100.4	40	0.0040
106.2	75.3	30	0.0030
70.8	50.2	20	0.0020
35.4	25.1	10	0.0010
17.7	12.5	5	0.0005
3.6	2.5	1	0.0001

In order to check these points a run was made using 30, 40, 50, 75 and 100 ppm (see Table 1 for molar and percentage values) over a period of 24 hours. A general scoring of the samples gave the following information: (1) 30 ppm produced *scatters* and almost no *clumps* even at the end of the run, 40 ppm produced a slightly larger number of clumps in a shorter period of time while all other doses ultimately produced nearly 100% *clumps*. All doses produced *scatters* initially. Since *scatters* can be produced indefinitely without *clumps* it was concluded that the former did not give rise to the latter but rather represented a *partial effect* (see HYPPIO, TSOU and WILSON, 1955). (2) Both time of initiation of effect and rate of change in effect were obviously dependent on dose. All slides were therefore scored in order to determine the times at which both *initial* and *full effect* were obtained. The *initial effect* time was designated as that when 10% of post prophases were deviant and *full effect* when 90—95% of post prophases were affected (see Table 2). It was later found, as will be noted, that a better definition of *full effect* could be made. When these times were plotted against dose, two approximately parallel curves were obtained, one representing *initial effect* and the other *full effect*. When plotted on log-log paper, the curves were rectified into straight line functions.

In order to study the cytological change in time with a given dose in more detail a run was set up with 40 and 50 ppm. In this case

temperature was rigidly controlled at 22.5° C and samples were taken at hourly intervals until *full effect* as represented by nearly 100%

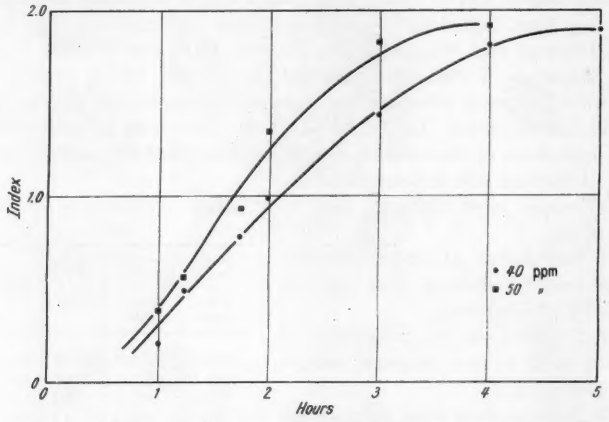

Fig. 2. Colchicine: Change in index of effect through time (40 and 50 ppm)

Table 2. *Best estimate of time that seedlings reached initial and full effect for various doses of colchicine*

Dose PPM	Time in minutes	
	Initial effect	Full effect
30	1260[1]	—
40	660	1260
50	480	780
75	150	300
100	60	120

[1] Uncertain.

Table 3. *Index of effect for 40 and 50 ppm colchicine*

Time in hours	Index of effect	
	40 PPM	50 PPM
1	0.20	0.39
1¼	—	0.57
2	1.00	1.35
3	1.44	1.84
4	1.83	1.93
5	1.92	—

Table 4. *Index of effect for 40, 50, 75 and 100 ppm colchicine*

Time in hours	Index of effect			
	40 PPM	50 PPM	75 PPM	100 PPM
1	—	—	0.06	0.09
2	—	—	0.32	1.00
3	—	—	0.46	1.40
4	—	—	0.81	1.57
5	—	—	1.01	—
6	—	0.24	1.46	—
7	—	0.47	—	—
8	—	0.28	—	—
9	—	0.57	—	—
10	—	1.01	—	—
11	—	0.88	—	—
12	0.21	0.87	—	—
13	0.19	1.07	—	—
14	0.22	—	—	—
15	0.38	—	—	—
16	0.34	—	—	—
17	0.47	—	—	—
18	0.23	—	—	—
19	0.51	—	—	—
20	0.52	—	—	—
21	0.88	—	—	—
22	—	—	—	—
23	0.85	—	—	—
24	1.13	—	—	—

clumped figures was reached. It may be noted that the rate of the reaction in this run was much faster than for equivalent doses in the

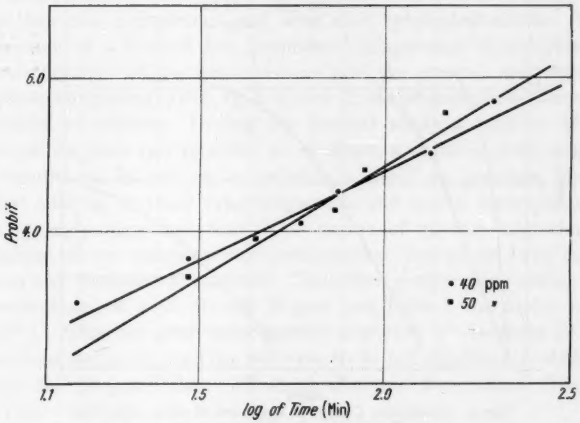

Fig. 3. Colchicine: Probit analysis line (40 and 50 ppm)

initial experiment. Such differences in rate apparently are due to temperature. In general the rate of the reaction appears to be very sensitive to temperature and, within limits, the higher the temperature the faster the reaction (see GOTOH, 1957). Since the colchicine reaction is characterized by two distinguishable configurations, some means of weighting these had to be devised. On the grounds that *scatters* appear first in time and are characteristic of low doses, it was assumed that they represented *partial effect*. We therefore arbitrarily assigned an index number of 1 to a *scatter* and 2 to a *clump* which gave us an index range of 0—2 for a complete reaction. When scored in this fashion the reaction curves in Fig. 2 are obtained (see Table 3). These appear to be sigmoidal and are rectified by plotting on probability paper or by applying probit analysis (Fig. 3). The sigmoid shape, however, probably reflects choice of scale rather than any fundamental property of the reaction itself.

Rescoring of the original experiment on the same basis gave the curves indicated in Fig. 4 (see Table 4). In most cases insufficient samples were available for the high end of the reaction curves. However, in so far as adequate scoring could be carried out the shapes of the curves are as expected. If the times at which individual dose curves cross arbitrarily chosen index values are plotted against dose (see Table 5), we

again obtain a series of statistically parallel curves which can be rectified on a log/log scale (Fig. 5). The general conclusions from these data are (1)

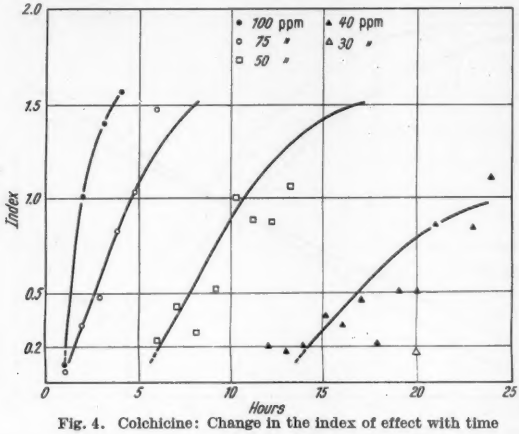

Fig. 4. Colchicine: Change in the index of effect with time

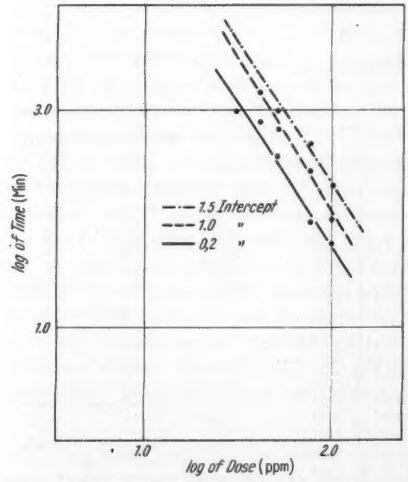

Fig. 5. Colchicine: Time-dose relationships for various dose intercepts

Table 5. *Best estimate of the time in minutes at which various dose curves pass through the intercepts*

Dose PPM	Time in minutes intercepts		
	0.2	1.0	1.5
30[1]	1200	—	—
40	860	1440	—
50	372	654	1020
75	96	282	480
100	66	120	212

[1] The 30 ppm dose is so close to the apparent threshold concentration that only the 0.2 intercept was measureable.

that *scatters* and *clumps* express different degrees of the same primary effect and (2) that the degree of effect is dependent on dosage which may be expressed as duration of exposure to a given concentration.

The actidione reaction

Previous work, especially that of HAWTHORNE and WILSON (1952) and BOWEN and WILSON (1954) have shown that actidione in sublethal doses has two conspicuous and consistant cytological effects: (1) The production of a deviant late prophase configuration characterized by over-contraction of the chromosomes and the gradual assumption of telophase morphology (Fig. 1 g, h, i) and (2) the progressive but reversible inhibition of mitoses. During the present study a number of pilot experiments were run in order to establish a suitable dose range for our conditions, as well as to provide a check on previous findings. Partial analysis of these runs fully confirmed earlier descriptions and very strongly suggested that both degree of mitotic inhibition and frequency of the characteristic configuration depend on both concentration and duration of exposure. Therefore a run was made using concentrations of 1, 5, 10 and 20 ppm (see Table 1 for molar values) at 22° C. This run was subsequently repeated to include 15 ppm. Since cross testing showed the two runs to be not significantly different, except for 15 ppm, data for final analysis were taken from the first run.

The major question which concerned us in the analysis was whether or not the two most conspicuous effects were reflections of the same basic action. In order to check this point we used two analytical techniques, one based on the deviant prophase (hereafter referred to as an actidione prophase) and the other on a function of mitotic inhibition.

Since actidione prophases are spatially late prophases, one measure of effect may be derived from the percent of late prophase configurations scorable as affected (see Table 7). This type of scoring gave the family of curves in Fig. 6. If dose-time plots are made for various levels of effect a series of statistically parallel curves rectifiable on log dose-log time scale is obtained (Fig. 9 A) (see Table 8).

Table 6. *Change in mitotic index through time for various doses of actidione*

Time in minutes	Concentration in PPM				
	1	5	10	15	20
0	48	48	48	48	48
60	39	47	49	29	36
120	53	40	29	25	33
180	34	34	27	17	25
240	28	15	22	16	16
300	21	—	—	—	—
420	21	—	—	—	—

While change in mitotic index might appear to be the most obvious measure of mitotic inhibition (see Table 6), the normal variation in mitotic activity usually makes it impossible to draw accurate enough curves without scoring an excessively large sample. While trend graphs did indicate

increase in rate of inhibition with increase in dose, a less variable index appeared to be desirable. We therefore chose to plot the ratio of early

Table 7. *Change through time in the number of actidione prophases expressed as percent of normal late prophases*

Time in minutes	Percent			
	5 PPM	10 PPM	15 PPM	20 PPM
0	0	0	0	0
45	—	—	34	35
60	19	31	—	—
90	33	55	60	77
120	56	76	73	84
180	72	91	94	98
240	75	—	—	—
300	98	—	—	—

Table 8. *Best estimate of the times at which the dose curves (% actidione prophases) cross the various intercepts*

Dose PPM	Intercepts in minutes	
	30 %	50 %
5	94	118
10	63	84
15	45	70
20	40	57

prophases to the rest of the division figures (E/R), a function which should measure inhibition but be little affected by variation in mitotic activity (see Table 9). This function will serve only if complete mitotic inhibition does

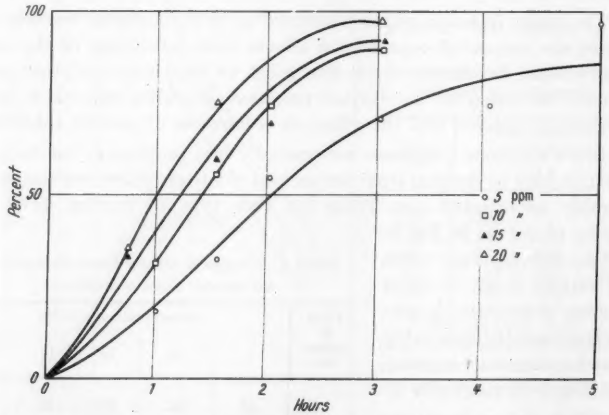

Fig. 6. Actidione: Change in the percent of actidione prophases/normal late prophases through time

occur. The resultant curves are given in Fig. 7 where it will be noted that 1 ppm does not completely inhibit at least during the period of the treatment. For the rest of the doses the ratio does fall to nearly zero. When the time at which dose curves cross arbitrarily chosen ratio intercepts are plotted against dose the curves for all intercepts are

statistically parallel and become straight lines when plotted on a log dose-log time scale (Fig. 9B; Table 10). Furthermore these curves do not differ significantly in slope from those obtained by using the actidione

Table 9. *Change through time in the ratio of early prophases to the total of all remaining mitotic figures*

Time in minutes	Ratio				
	1 PPM	5 PPM	10 PPM	15 PPM	20 PPM
0	1.44	1.44	1.44	1.44	1.44
60	1.10	1.26	1.20	1.33	0.81
75	—	—	—	0.71	0.55
90	—	—	0,81	—	—
105	—	—	—	0.48	0.35
120	0.94	1.13	0.43	0.30	0.13
150	—	0.67	—	—	—
180	0.60	0.46	0.09	—	—
240	0.52	0.23	—	—	—
360	0.63	—	—	—	—

Table 10. *Best estimate of the time at which the dose curves (early prophase/total remaining figures) cross the various intercepts*

Dose PPM	Intercepts in minutes		
	0.2	0.3	0.5
5	240	202	170
10	159	142	115
15	140	120	95
20	116	101	81

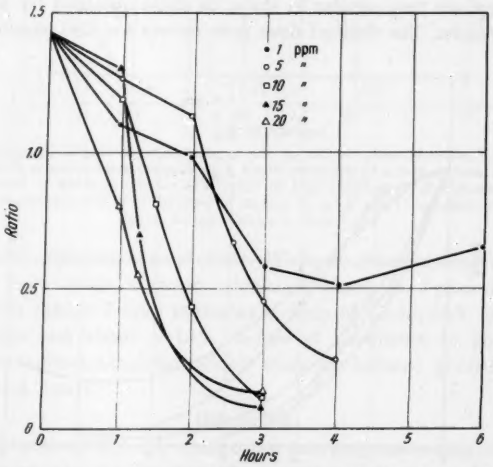

Fig. 7. Actidione: Change in the ratio of early prophases/total of all remaining mitotic figures with time (E/R)

prophase as a basis of measurement. This would seem to indicate that mitotic inhibition and production of the actidione prophase result from primary effects on the same mechanism. Also these results suggest that this susceptible mechanism is present from interphase to at least late prophase.

A third type of measurement, which in part reflects both effects is the ratio of early to late prophases (E/L) (see Table 11). The curves

Table 11. *Change through time in the ratio of early prophases to late prophases*

Time in minutes	Ratio				
	1 PPM	5 PPM	10 PPM	15 PPM	20 PPM
0	7.60	7.60	7.60	7.60	7.60
60	6.20	6.80	4.00	—	3.50
90	—	—	—	2.50	—
105	—	—	—	1.67	—
120	4.80	3.80	2.00	1.48	1.20
150	—	—	—	0.76	—
180	2.80	1.90	0.38	—	0.21
240	1.80	0.47	0.10	—	0.20
420	2.20	—	—	—	—

Table 12. *Best estimate of the time at which the dose curves cross the various intercepts (E/L)*

Dose PPM	Intercepts in minutes		
	1	2	3
5	211	174	142
10	153	108	78
15	139	100	70
20	129	93	68

derived from this type of measurement are shown in Fig. 8. As expected they are very similar in shape to those obtained by measuring inhibition alone. The derived dose time curves are also parallel to each

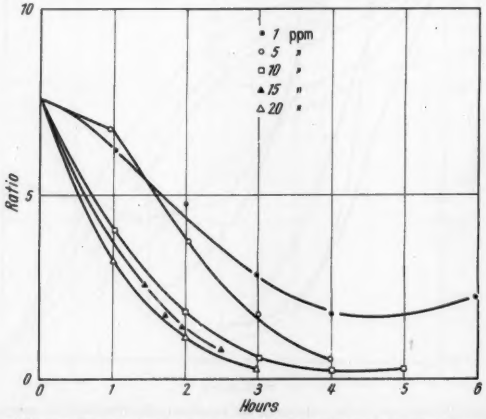

Fig. 8. Actidione: Change in the ratio of early prophases/late prophases with time

other and to those obtained from the other two types of measurement (Fig. 9C; see Table 12).

The general conclusion therefore is that measurements based either on inhibition or frequency of actidione prophase or a combination of both measure the same fundamental action.

The main value of the E/L measurement is the information which it gives concerning partially effective doses. It will be noted (Fig. 8) that after 1 ppm treatment the value drops to a new level and becomes stabilized. This is expected only if there is no or only partial inhibition of mitosis combined with at least a partial shunt of potential post prophases into the actidione prophase configuration. Knowledge of mitotic index change combined with E/L and E/R data allows us to make

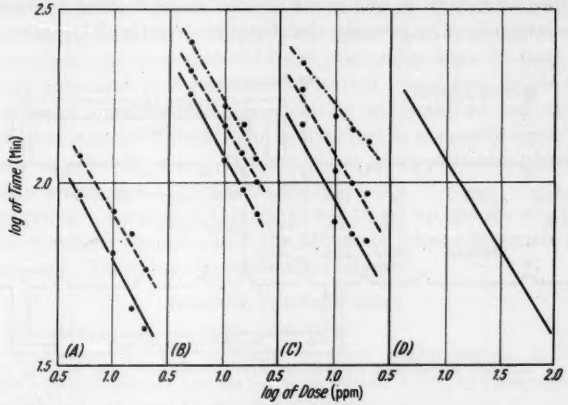

Fig. 9. Actidione: Linear relationships of all types of measurements. *A* Actidione prophases as % of normal late prophases. *B* Early prophase as a ratio of total of remaining mitotic figures. *C* Early prophases as a ratio of late prophases. *D* Common slope for all types of measurements. The families of curves in *A*, *B* and *C* represent various effect levels (comp. Tables 8, 10 and 12)

quantitative estimates of total effect. While no detailed analysis of this type has been made, a rough calculation based on 1 ppm data after stabilization (about 7 hrs.) indicates a drop of about 50% in onset of new mitoses and about a 15% failure of prophases to go through normal metaphase-anaphase which state of balance persists for at least several hours.

Discussion

The data presented clearly confirm our previous statements concerning the two reactions, namely; that they are very different and that they are highly specific. A diagrammatic representation of the two reactions is given in Fig. 10. The action of the drugs is represented by "valves" at the points at which they are postulated to work. Thus if "valve 3" is pushed down (representing colchicine effect), normal movement halts and morphological transformation continues *in situ*. This is represented by the detour marked colchicine pathway. This detour results in either polyploidy or multinucleated cells depending on the degree of effect.

The actidione action is represented by two "valves". Closing the first one represents the inhibitory action, while closing the second, represents the production of the actidione configuration and the detour marked prophase poison represents inhibition of the movement cycle with morphological transformation taking place *in situ*.

Little more need be said concerning the colchicine reaction. By and large the results are in full agreement with those previously reported from this laboratory as well as with those of most other workers. So far as comparison is possible the dose-time relationship agrees with

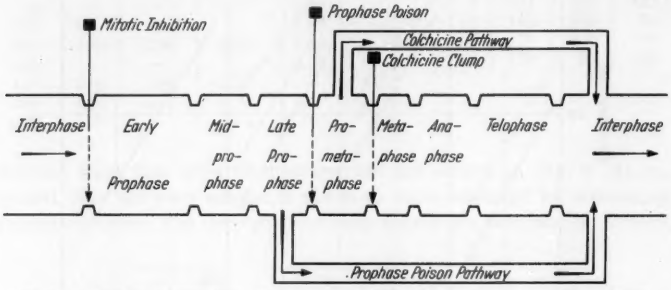

Fig. 10. Diagram representing interference with mitotic movement cycle in *Pisum*

LEVAN (1954). One fact that should be stressed is that both scatter and clump configurations represent different degrees of the same kind of action.

Why *partial effect* should result in *scatters* is not completely clear. One possible explanation is derived from the following consideration. If the spindle develops from a small spherical precursor by a process of swelling and dipolar organization then *scatters* could arise from partial or even complete swelling of the precursors before the chromosomes had reached the prometaphase position and thus they would be physically prevented from clumping. The degree of *scatter* would depend on the degree of inhibition and the higher the dose the less wide the scattering which in general agrees with observations.

The results obtained from the actidione studies also do not differ significantly from those previously reported. As noted by BOWEN and WILSON (1954) it is clear that the prophase poison action differs markedly from a c-mitotic action with an entirely different mechanism being affected. Also the reaction is obviously just as specific and the two most conspicuous effects, namely mitotic inhibition and the actidione prophase figure reflect the same action. What the system or structure affected may be is a matter on which we can only speculate at the

moment. There is, however, some reason to suspect involvement of the nuclear membrane or wall or both. In the first place an increase in nuclear volume by a factor of about 1.5 is associated with the inception of prophase in pea roots (WILSON and HYPPIO, 1955; M. E. HAWTHORNE, unpub.) and the typical actidione prophase is characterized by apparent retention of a limiting wall through to the next interphase (BOWEN and WILSON, 1954). Since expected changes do not occur in the nuclear boundary in treated tissue, specific alteration of its normal properties seems rather probable. Indeed, with regard to the changes at late prophase they may be considered to be irreversible since we have never seen any indication that resulting restitution nuclei ever divide again.

Studies of low doses of both colchicine and actidione indicate that *partial effect* may be obtained and maintained in a state of equilibrium for some considerable time. This is at least of theoretical interest with regard to action of anti-neoplastic agents.

The primary purpose of this study was to set up specific criteria for the two reactions against which the effects of other compounds might be compared. These may be outlined as follows:

Colchicine (c-mitotic agent)

1. No inhibition of the onset of mitosis.

2. Production of the following deviant configurations in order: a) *scatters* (c-metaphase) associated with *partial effect*; b) *clumps* (stalled prometaphase) associated with *full reaction*.

3. For a given dose under standard conditions there should be a steady increase in the degree of effect with time until either full effect or an equilibrium is reached.

4. If the times at which several dose curves cross arbitrarily chosen effect levels are plotted on a log dose/log time scale a series of straight and parallel lines should be obtained.

5. Polyploid or multinucleate cells or both should be recoverable.

Actidione (prophase poison)

1. The onset of mitosis should be inhibited.

2. A specific deviant figure with the spatial characteristics of late prophase should be produced (Fig. 1g, h, i).

3. Measurements based on either inhibition or the deviant figure should indicate a steady increase in effect with time for any given effective dose.

4. If the times at which various dose curves pass through arbitrarily chosen effect intercepts are plotted on a log dose/log time scale a group of parallel straight lines should be produced.

5. Following recovery divisions figures are expected to be normal in chromosome number and constitution.

Summary

1. The *Pisum* test was used in an endeavor to set up cytological criteria for c-mitotic and prophase poison actions on a qualitative and quantitative basis. Colchicine was chosen as the standard type c-mitotic agent, and actidione as the standard type prophase poison.

2. The effects of colchicine and actidione as reported by previous investigators were confirmed. By assigning weights to the configurations obtained with colchicine, and by measuring the change in the ratio of configurations and the configurations themselves plus a combination of the two in the case of the actidione, a linear relationship between time and dose is obtained.

3. A series of cytological characteristics are suggested which a drug or chemical should satisfy to fall within the classification of a c-mitotic agent or a prophase poison.

4. The possible involvement of the nuclear membrane and/or wall in prophase poison action is suggested.

5. The fact that c-mitotic reactions and prophase poison actions are clearly and distinctly separable reactions is re-emphasized.

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POLYPLOIDY AND DIFFERENTIATION IN THE TRANSITIONAL EPITHELIUM OF MOUSE URINARY BLADDER

By

BRUCE E. WALKER

With 9 Figures in the Text

(Eingegangen am 25. November 1957)

Introduction

Transitional epithelium of urinary bladder in rodents is characterized by a surface layer of very large cells which, during division, display unusually large mitotic figures (FLEROFF 1936; VULPĚ 1954). LEUCHTENBERGER, LEUCHTENBERGER and DAVIES (1954) found multiple quantities of DNA (deoxyribonucleic acid) in nuclei of human urinary bladder; and WALKER (1955) described polyploid divisions in mice in a preliminary report of chromosome counts from squash preparations of transitional epithelium. The first objective of this report is to discover which cell types in transitional epithelium are polyploid.

The question of whether genic material differentiates during ontogeny has not been settled. The possibility that polyploidy (as a form of nuclear differentiation) influences cytoplasmic differentiation has been discussed by HUSKINS (1947, 1952) and others (WHITE 1945, SWIFT 1950) and reviewed in detail by SCHULTZ (1952). Investigation of the relationship, if any, between polyploidy and differentiation in transitional epithelium is the second objective of this project. The third objective is to study the role of growth hormone in the polyploidy of transitional epithelium, since HELWEG-LARSEN (1952) and LEUCHTENBERGER, HELWEG-LARSEN and MURMANIS (1954) have demonstrated that lack of growth hormone can prevent the formation of large (polyploid) nuclei.

Materials and methods

A stock of hybrid albino mice was used for most of the experiments. Heterozygous mice[1] carrying the *Sd* (Danforth's short tail) or *dw* (pituitary dwarf) mutations were used for the remaining experiments. The age of embryos was estimated from vaginal plug timing and from the morphological criteria of GRÜNEBERG (1943). Urinary bladders were fixed in Bouin's or Carnoy's fluid, sectioned at 6μ, and

[1] Courtesy of Dr. M. N. RUNNER and Dr. G. D. SNELL of the Roscoe B. Jackson Memorial Laboratory.

stained with hematoxylin and eosin. Nuclear and cell diameters were measured with an ocular micrometer and the results expressed as an average of the major and minor axes at right angles to each other. Each average in Table 1 is based on 200 nuclei and in Table 2 on 100 nuclei and 100 cells. The relative distance of a nucleus from the lamina propria (Table 1) was calculated by dividing the thickness of the epithelium at that point into the distance of the centre of a nucleus from the lamina propria.

Table 1. *Characteristics of nuclei in transitional epithelium of mouse bladder*

Nuclear characteristics		Age of mouse		
		newborn	3 weeks	adult
Distribution of nuclear diameters (μ)	$\bar{x}$	7.31	7.24	7.79
	σ	1.85	2.01	2.04
	S_K	1.06	1.05	0.88
Correlation of nuclear diameter with distance from lamina propria	r	0.345	0.676	0.511
	p	.001	.001	.001

To prepare slides for chromosome counting, bladders were excised, pretreated one minute with water (HSU and POMERAT 1953), and fixed 5 minutes in alcohol-acetic (3:1). The inner surface of each bladder was scraped onto a slide, covered with a drop of aceto-carmine, lightly flattened with a cover glass, flooded with Wilsons' Venetian Turpentine, then heavily flattened (WALKER and BOOTHROYD 1954). Counts were made through the phase contrast microscope with and without preliminary sketching of the chromosome groups.

Bladders used for microspectrophotometry were fixed in alcohol-acetic (3:1), sectioned at 12μ to 25μ, and stained by the Feulgen reaction or with periodic acid-acriflavine-Feulgen (HIMES and MORIBER 1956). In the latter procedure, tissue sections were hydrolyzed 12 minutes ,stained with the Feulgen reagent, treated with periodic acid 5 minutes, stained with acriflavine 5 minutes, bleached four minutes, and mounted in oil with a refractive index of 1.56. Microspectrophotometric measurements were made in Dr. A. W. POLLISTER's laboratory, with the help of Dr. MARION HIMES, using apparatus of the type described by POLLISTER (1952 — Fig. 2, p. 108). The relative amount of DNA per nucleus was estimated by measuring transmittance through a central core in each nucleus and calculating the total amount of dye by treating the nucleus as a sphere, as described by SWIFT and RASCH (1956).

Results

Nuclear size

Nuclei in the transitional epithelium of mouse urinary bladder display a wide range of sizes (Figs. 2, 3). When average nuclear diameters ($\bar{x}$, Table 1) are plotted they form a continuous distribution with only a single pronounced peak. However, the distribution is strongly skewed to the right ($S\bar{k}$, Table 1). These characteristics of nuclear diameters are established in the newborn and do not change significantly with increased age (Table 1). Nuclei appear to be smallest near the lamina propria and largest at the epithelial surface (Figs. 2. 3). Analysis of the

association between nuclear diameters and the relative distance of each nucleus from the lamina propria gave significant correlations (Table 1). Nuclear diameters increase as the relative distance of nuclei from the lamina propria increases.

DNA content of nuclei

DNA measurements of nuclei in transitional epithelium fall into two main classes and a smaller third class, averaging approximately 2, 4, and 8 arbitrary units, respectively (Fig. 1a). The DNA content of a nucleus was found to be closely correlated with its diameter ($r = 0.94$).

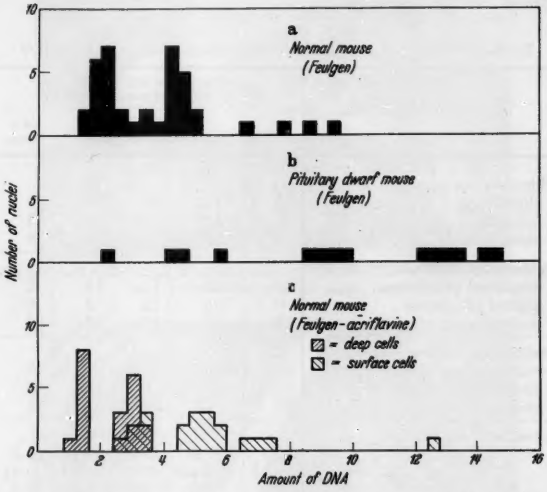

Fig. 1a—c. DNA content of nuclei from transitional epithelium of mouse urinary bladder

In this series of measurements, the Feulgen stain alone was used, and surface cell nuclei could not be differentiated from deep cell nuclei.

When periodic acid-acriflavine is used to stain cytoplasm, it is possible to distinguish surface cells from deep cells (Vulpě 1954, Mende and Chambers 1957), and this technique was used to measure DNA in 20 nuclei of each cell type. Three main classes can be recognized (Fig. 1c), but the average values of these classes — approximately 1.4, 3, 5.6 — are lower than when the Feulgen stain alone is used. The nuclei of deep cells gave values that fell into the first two classes, whereas the nuclei of surface cells all had values higher than those of the lowest class (Fig. 1c).

DNA was also studied in the transitional epithelium of a pituitary dwarf mouse, with the large nuclei being selected for measurement. These large nuclei contained a high amount of DNA (Fig. 1b).

Histogenesis

In $14^1/_2$ day old embryos, the epithelium lining the bladder lumen is multilayered, with all the cells and their nuclei being about the same size (Fig. 4). The epithelium looks much the same on the following day except for the presence of a few binucleate cells in the surface layer. Chromosome counts from squash preparations of the epithelium gave only diploid values (Table 2).

Table 2. *Histogenesis of transitional epithelium in mouse bladder*

Cell characteristics	Approximate age of embryo (days after conception)				
	$15^2/_3$	$16^2/_3$	$17^1/_3$	$18^2/_3$	newborn
Chromosome counts					
diploid	45	31	10	10	68
tetraploid	0	10	6	0	63
octoploid	0	1	1	4	8
Surface cell mitoses					
unpaired prophases	18	45	7	19	2
paired prophases	0	100	12	2	6
metaphases, anaphases	14	59	17	19	12
Deep cell mitoses					
unpaired prophases	66	188	40	87	46
paired prophases	10	15	8	10	5
metaphases, anaphases	52	184	20	53	44
Average diameters (μ)					
surface cells $\bar{x}$	12.7	17.9	17.3	17.1	24.0
$S.E._{\bar{x}}$	0.17	0.44	0.36	0.34	0.44
surface nuclei $\bar{x}$	7.0	8.3	8.7	8.2	9.2
$S.E._{\bar{x}}$	0.07	0.13	0.15	0.11	0.14

The transitional epithelium of $16^2/_3$ day old embryos displays fundamental advances over the preceding day. Polyploid mitotic figures are plentiful in squash preparations, and the average diameter of nuclei and cells shows a pronounced increase (Table 2, Fig. 6). Binucleate surface cells occur with a high frequency over a considerable proportion of the epithelium (Fig. 5), and more than two-thirds of the prophases in surface cells are in pairs (Table 2). Less than 10% of the prophases in deep cells occur in pairs. In the deep cell layer, it is not clear whether each pair of prophases is always in a single cell, because the cells are crowded and poorly defined compared to the surface cells. The later stages of mitosis (metaphases, anaphases — Table 2) were not paired,

but appeared as if they were derived from the division of a mononucleate cell. The characteristics originating in the $16^2/_3$ day old embryos

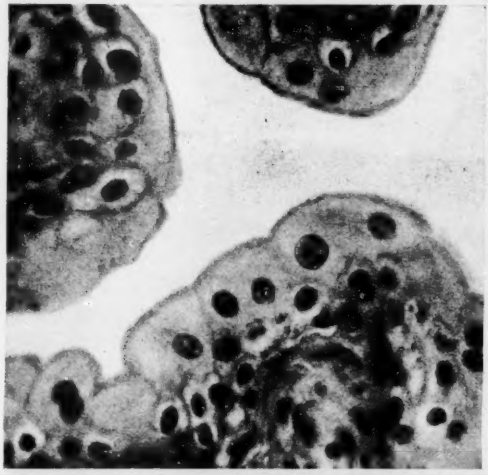

Fig. 2. Transitional epithelium of mouse urinary bladder. The surface cells are very large and form a layer one cell thick. 540 ×

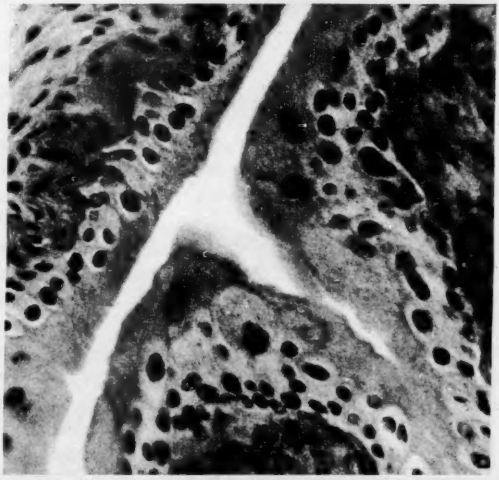

Fig. 3. Transitional epithelium stained with periodic acid-Schiff-hematoxylin. The surface cell cytoplasm stains with the Schiff reagent, it forms a continuous lining. 380 ×

continue without significant change throughout the remainder of fetal development (Table 2).

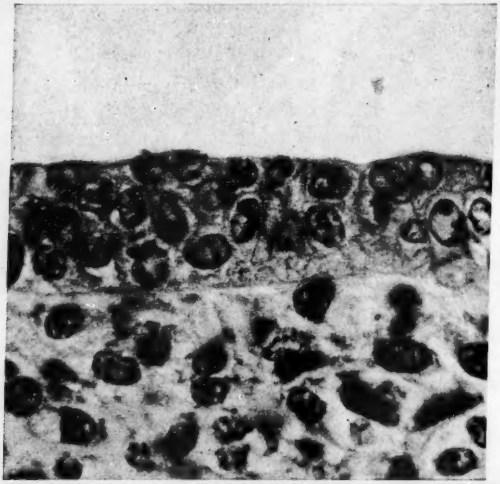

Fig. 4. Bladder epithelium from a 14½ day old mouse embryo. The cells and nuclei are uniformly small. 860 ×

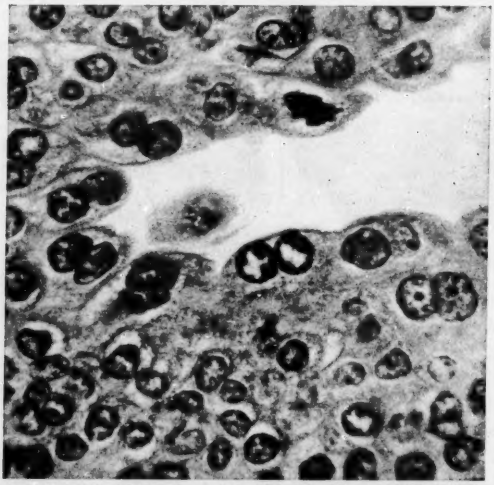

Fig. 5. Epithelium lining the bladder lumen of a 16½ day old embryo. Note the high frequency of binucleate cells in the surface layer, and the pair of prophases in centre field. 860 ×

After birth, a second increase in the average diameter of nuclei and cells takes place, and this brings the surface cell to its final stage of

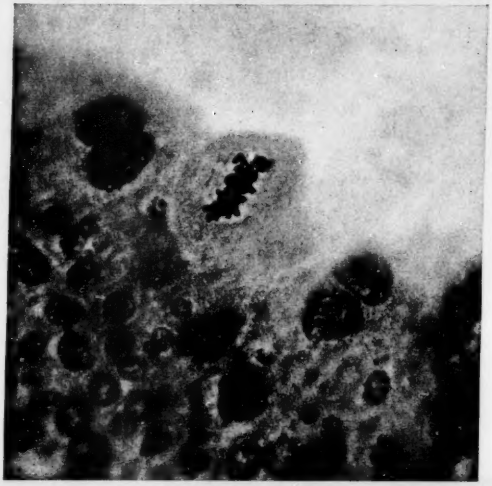

Fig. 6. Transitional epithelium of a 16½ day old embryo from an area showing large surface cells and nuclei. In centre field is a typical large, single metaphase. 860 ×

differentiation regarding nuclear and cell size (Fig. 7). The most extensive survey of chromosome numbers was performed on newborn mice (12 to 36 hours post partum), and it was found that about half of the mitotic figures were tetraploid or octoploid (Table 2 and Figs. 8, 9). This may represent some selection in favor of large groups, since they are more readily located on the slide. Squash preparations include all of the epithelium so the chromosome counts represent both surface and deep cells.

Although the chromosome counts are reported as exactly diploid, there were small deviations which can be attributed to errors in counting (WALKER and BOOTHROYD 1954). There is evidence for (THERMAN and TIMONEN 1951, HSU and PEMERAT 1953, TONOMURA and YERGANIAN 1957) and against (WALKER and BOOTHROYD 1954, TJIO and LEVAN 1956) the existence of aneuploidy, but if aneuploid chromosome groups exist in transitional epithelium, they do not differ from euploidy sufficiently to be distinguishable from counting error.

Lack of growth hormone apparently has no effect on polyploidy in transitional epithelium after the polyploidy has become established. Comparison of nuclei in transitional epithelium from a pituitary dwarf

mouse and one of its littermates showed no significant difference in the average nuclear diameters ($t = 0.329$, d. f. $= 398$, $p > 0.7$). Also, DNA

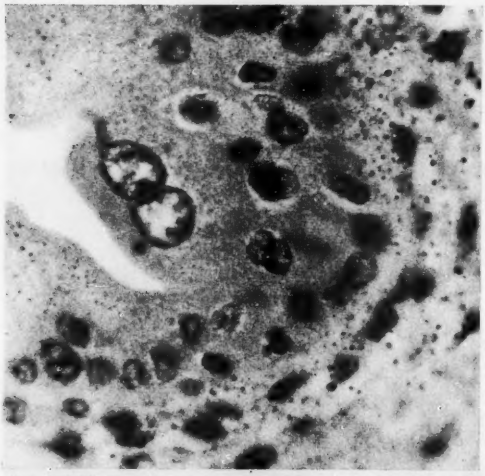

Fig. 7. Transitional epithelium from a newborn mouse, stained with periodic acid-Schiff-hematoxylin. A pair of prophases can be seen in a giant surface cell. Each prophase is probably polyploid, judging from its size. 860 ×

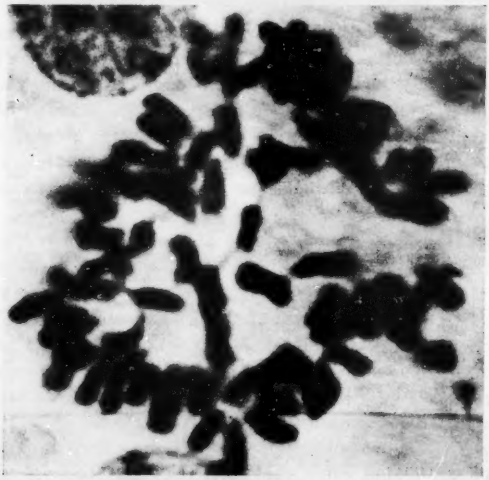

Fig. 8. A group of chromosomes from a squash preparation of transitional epithelium. There are about 80 chromosomes present. approx. 3000 ×

values in the transitional epithelium of a pituitary dwarf mouse ran as high as in normal mice, as described above (Fig. 1 b).

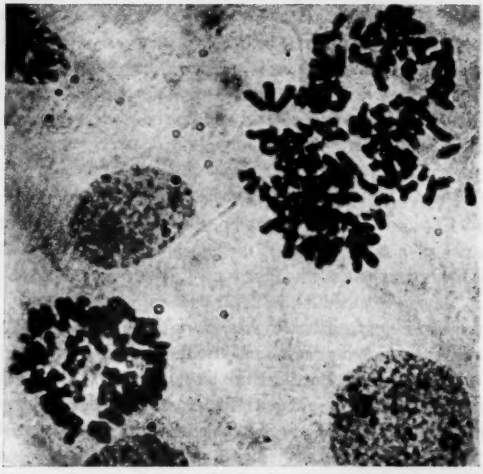

Fig. 9. Two chromosome groups from a squash preparation of newborn mouse transitional epithelium. The group in the lower left is tetraploid and the other larger group is octoploid. approx. 1200 ×

The possible involvement of the metanophros in initiation of polyploidy in the bladder was investigated by mating mice with the *Sd* (Danforth's short tail) mutation and studying the late fetus and newborn. A small percentage have no metanephroi (GLUECKSOHN-SCHOENHEIMER 1943), and the transitional epithelium of several such mice was examined. No differences from the normal in nuclear diameter or chromosome number have been detected so far.

Discussion

Location of polyploid nuclei

The most direct evidence concerning the distribution of polyploid nuclei comes from DNA measurements. All the surface cell nuclei measured had quantities of DNA that were multiples of the minimum amount found in deep cells (Fig. 1 c) and were, therefore, polyploid. The amount of DNA in deep cells fell into two classes (Fig. 1 c), so some deep cell nuclei must be polyploid also. Evidence as to the distribution of these polyploid deep cells can be obtained from consideration of nuclear diameters, since the latter were found to be well correlated with DNA

content. Nuclear diameters increased with increased relative distance of the nuclei away from the lamina propria (Table 1), so the tetraploid deep cells are probably mainly or entirely in the outermost layer of deep cells. (Also, the decreased overlapping of nuclei in the outer layers compared to the layer adjacent to the lamina propria results in a tendency to select nuclei from the former for microspectrophotometric measurements.) A population of polyploid deep cells directly under the surface cells might be a source of replacement for surface cells lost into the bladder lumen.

Influence of growth hormone

HELWEG-LARSEN (1952) showed reduction of nuclear volume in certain tissues of pituitary dwarf mice, as compared to normal littermates, and LEUCHTENBERGER, HELWEG-LARSEN and MURMANIS (1954) reported an "absence of multiple DNA classes" in these mice. However, in the transitional epithelium of bladders from pituitary dwarf mice, there is no significant change in nuclear diameters, and DNA values are as high as in normal mice (Fig. 1 b). Polyploidy in transitional epithelium differs from that of other somatic tissues known to have polyploid nuclei in that the polyploidy is well established before signs of growth hormone deficiency appear in pituitary dwarf mice. Growth hormone is not necessary for maintenance of polyploidy, but it may be involved in the initiation of polyploidy during the histogenesis of transitional epithelium, since there is evidence of pituitary gland activity in the embryo (FRANCIS 1944, JOST 1957, SMITH and DORTZBACK 1929).

Origin of polyploidy

Polyploidy arises in the transitional epithelium of the embryo at approximately $16^2/_3$ days after conception (Table 2). It is correlated with the appearance of a large number of binucleate cells and by the occurrence of pairs of prophases in surface cells (Fig. 5). Since metaphases (Fig. 6) and anaphases are single, it is concluded that the prophase pairs fuse at late prophase and form a single metaphase plate. A normal anaphase would then lead to the formation of two tetraploid nuclei. If the nuclei in a binucleate cell were both tetraploid (Fig. 7), the division product of fusion during prophase would be two octoploid cells. The phenomenon of nuclei in a binucleate cell entering division synchronously is seen from day $16^2/_3$ to birth. The figures for division frequency in newborns (Table 2) give the impression that divisions had become infrequent in surface cells as compared to deep cells; but this is due, at least in part, to the very large size of the surface cells at birth, and the consequent high ratio of deep cells to surface cells in a given area.

Since polyploid surface cells of embryonic transitional epithelium are smaller than the same cells in postnatal epithelium, and since polyploid nuclei occur in some of the deep cells — which are considerably smaller than surface cells, therefore the large size of postnatal surface cells is probably not due to polyploidy alone. Yet it is equally probable that the surface cells could not reach their exceptionally large size and function efficiently (BERRILL 1955) unless their nuclei were polypolid. Thus, the conversion to polyploidy in the embryo would anticipate the need at birth for extra chromosomes that would enable the cells to respond to a factor promoting excessive cell size (i.e., a case of prophthasis — REYNOLDS 1954). In summary, polyploidy can be considered as one of the essential factors, though not the only one, in the differentiation to large size of the surface cells.

Genetic differentiation of somatic nuclei

Within the nucleus reside the factors that guide the growth and differentiation of an organism in a manner specific to its heritage. In considering how this is brought about, one of the most basic questions is: Do somatic nuclei remain undifferentiated? or do they set the final characteristics of somatic cells by becoming differentiated themselves?

Concerning differentiation in a general sense (WEISS 1953) there is abundant evidence of nuclear differentiation on the basis of enzyme content (ALLFREY, MIRSKY and STERN 1955) and nuclear morphology (e.g. polymorphonuclear leucocytes). However, to implicate a "genetic" differentiation comparable to that which operates at the whole organism level, there should be evidence of relatively stable changes involving the genic material specifically.

One possible criterion of such a nuclear change concerns the ability of a nucleus to guide the development of an egg to maturity. SPEMANN (1938) found that blastula nuclei could effectively replace the egg nucleus, and more recently, KING and BRIGGS (1954) showed, with nuclear transplants, that most nuclei up to the late gastrula stage are totipotent. However, the latter authors now have evidence (BRIGGS and KING 1957) of relatively permanent differentiation in nuclei of post-neurula endoderm.

Another criterion of genetic differentiation is morphological change of the chromosomes. BEERMANN (1956) has offered, as evidence of nuclear differentiation, the puffs and rings of Balbiani on giant *Diptera* chromosomes. Another morphologically identifiable change of somatic chromosomes is polyploidy. Its genetic effects are of sufficient magnitude to produce readily observable differences in phenotype (e.g. autotetraploid plants — SINNOTT et al. 1950, p. 368; polyploid salamanders — FANKHAUSER 1945), and of sufficient stability to become distributed

through all the cells of the organism if the polyploid condition arises in the egg (FANKHAUSER 1945). Somatic polyploidy has long been known and its possible relation to cytoplasmic differentiation frequently discussed (HUSKINS 1947, 1952; WHITE 1945; LEUCHTENBERGER, HELWEG-LARSEN and MURMANIS 1954). However, one of the difficulties in correlating polyploidy with cytoplasmic differentiation has been that the polyploidy arose at the same time as cytoplasmic differentiation (SCHULTZ 1952) or even later (SWIFT 1950) and therefore may have been the result, rather than the cause, of the cytoplasmic differentiation. Yet, if polyploidy cannot be established as a case of nuclear differentiation influencing cytoplasmic differentiation, then the possibility of identifying morphologically a genetic differentiation of the somatic nucleus becomes rather remote. There are three reasons for this conclusion. Firstly, of the conceivable forms of genetic differentiation in the nucleus, polyploidy is the easiest to identify (at metaphase) and follow (as DNA). Secondly, its genetic effects are not radical and so it should be compatible with the overall genetic integrity of the animal. Thirdly, polyploidy is so readily caused by a variety of agents and circumstances, that it should have occurred frequently in somatic tissues during evolution and thus provided ample opportunity for its causative agents to have been established in the genotype by natural selection.

There are several reasons for believing that the polyploidy in transitional epithelium of the mouse is a case of nuclear differentiation influencing cytoplasmic differentiation:

1. It arises early in ontogeny, at a time when natural selection would be expected to have a pronounced effect; therefore, this case of polyploidy is not likely to be a degenerative or indifferent phenomenon.

2. Its consistent localization and timing (i.e. — surface cells of $16^2/_3$ day old embryos — Table 2) indicates that this is a well controlled mechanism of embryological development.

3. In contrast to previously known cases of somatic polyploidy, it clearly precedes the characteristic differentiation of the cells, since the unusually large size of the surface cells and nuclei is not attained until after birth.

4. There is good reason to correlate the nature of the nuclear change with the nature of the cytoplasmic change. The most consistent effect of polyploidy is to produce large cells (FANKHAUSER 1954). It is not known why the surface cells in transitional epithelium of the mouse bladder are so large, although the theory that such a cell can stretch more efficiently than a small cell seems plausible. However, whatever its purpose may be, the large size of the surface cell is its outstanding characteristic, and the evidence presented here supports the hypothesis that polyploidy is an essential factor in the production of this characteristic.

Summary

1. There is a wide range in the size of nuclei from transitional epithelium of mouse urinary bladder, with the largest nuclei being nearest the lumen. These characteristics are present at birth and do not change significantly thereafter.

2. Microspectrophotometric measurements of nuclear DNA fall into several classes, indicating the presence of polyploidy. All surface cells and some deep cells are polyploid.

3. Neither the amount of DNA per nucleus nor the distribution of nuclear diameters is significantly altered in pituitary dwarf mice.

4. The counting of chromosomes in squash preparations of transitional epithelium showed the presence of polyploid chromosome groups in the mouse embryo from day $16^2/_3$ to birth.

5. The appearance of polyploidy in embryonic transitional epithelium was paralleled by the appearance of many binucleate cells, the nuclei of which were seen to enter division synchronously. The widespread presence of two prophases in a single cell and the lack of such duality in the later stages of division was taken as evidence of polyploid formation through fusion at late prophase and subsequent normal division into two polyploid nuclei.

6. The manner in which polyploidy was involved in the histogenesis of transitional epithelium led to the hypothesis that one characteristic of cytoplasmic differentiation in surface cells was brought about, at least in part, by nuclear differentiation.

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THE PATTERN OF PROTEIN SULPHUR AFTER FEULGEN HYDROLYSIS IN THE SALIVARY GLAND CHROMOSOMES OF DROSOPHILA MELANOGASTER

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With 19 Figures in the Text

(Eingegangen am 25. November 1957)

The salivary gland chromosomes of Diptera stand in a category by themselves in respect of the conclusiveness of the cytogenetical work conducted on them, and the cytochemical information about them is increasing parallel to the refinement of techniques. The interest of the cytogeneticist arose from the easily observable and stable pattern of DNA, shown as bands in the classical maps of the BRIDGES, which was taken to represent the genetical organisation of the polytenic chromosomes. Staining for compounds other than DNA results in a banding pattern different from that due to stains for DNA (ALFERT 1954; PAVAN and BREUER 1955). By tracer technique it was found that most, but not all, DNA bands are the site of an active RNA metabolism (PELC and HOWARD 1956), a fact that was predictable from the staining of the chromosomes for both nucleic acids, which shows superposition of both nucleic acids, and from the known active metabolism of nuclear RNA. The distribution in eu- and heterochromatin, and bands and interbands of nucleic acids, proteins and their components, as well as minerals, and the structural organisation of the chromosomes, have been reviewed by ALFERT (1954) and RIS (1957).

The present investigation deals with the pattern of protein sulphur in the polytenic chromosome, as revealed by incorporated isotope. It was hoped that this approach would permit the quantitation of amounts of sulphur beyond the sensitivity of staining procedures, and their relation to the microscopical topography of the chromosome. A recent cytophotometric procedure determines the ratio of SH to SS-groups (TEIGER, FARAH and DI STEFANO 1957). In *Drosophila*, a joint pattern of NH_2 and SH groups has been shown using coupled stains (DANIELLI 1949), but, owing to the abundance of the former, it was impossible to decide the pattern of SH. It has, however, been shown that the labelling with radiomethionine is of uneven intensity along the chromosomes (PELC and HOWARD 1956).

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Material and techniques

One milligram of DL-methionine-^{35}S (132 μc) was dissolved in 2 cc distilled water and mixed into 50 cc Drosophila food (corn meal-treacle-yeast) under semi-sterile precautions. Ten pairs of adult flies (Ore-R strain) were seeded for 4 days on the food at 25° C. From day 6 the culture was kept at 12° C and from day 12 at 18° C, to avoid overcrowding and to obtain larvae of large size. The larvae approaching pupation, used for study, were collected at day 14, after they had been feeding on the radioactive food for 9—13 days; previous collections were used for procedural tests. Only one generation of larvae were grown on radioactive food and the resulting flies were mass-subcultured in normal food for several generations.

Female larvae were rinsed free from food and the salivary glands were dissected and squashed under cellophane in carmine-45% acetic acid, rinsed (SLIZYNSKI 1952); hydrolysed in N HCl at 60° C for 5 minutes, and stored overnight either before or after hydrolysis in 50% alcohol. The squashes were prepared on albuminized slides previously rimmed and backed with gelatin-alum, to ensure adhesion of the autoradiographic emulsion; it was found that squashes did not adhere well to gelatinized slides usually used for autoradiography, and that their cytological appearance was probably not quite as good as when prepared on albuminized slides. The presence of carmine was preferred to secure proper squashing. This stain at acid pH's behaves like a basic dye (BAKER 1945); it will be expected to combine sooner with nucleic acids than with proteins, and therefore not to interfere much with tracer in the proteins, i.e. by displacement. To minimize any such latter action of the stain a concentration was used which is half that used in routine cytological work, the stain was not an iron lake and the stay in aceto-carmine did not exceed fifteen minutes. Squashes for the scoring of chromosomal mutations (see below) were prepared by routine methods (SLIZYNSKI 1949, 1952). Chromosomes of radioactive larvae sampled at day 21 appeared normal in routine squashes, as did the chromosomes sampled at day 14 for autoradiography.

The slides were rinsed in distilled water for more than one hour, and coated in a single run with Kodak stripping film stretched at a maximum. All films were simultaneously exposed in light-tight plastic boxes for three days at 5° C, developed for three minutes in Kodak D 19b, and fixed for 10 minutes in 1/10 Fix-Sol (all processing carried out at 17.5° C). Low grain size (0.3 μ) and density were essential. After several hours of rinsing the preparations were stained with basic fuchsin for 45 minutes, bleached for two minutes in SO_2 water, quickly dehydrated, and mounted with Euparal (SLIZYNSKI 1949). This schedule proved the nearest to a satisfactory staining of the chromosomes with a minimum staining of the emulsion. Dry-mounting resulted in lesser clarity of the chromosomes. Fully mature chromosomes were studied from a final batch of twenty-three squashes.

Autoradiographs (ARGs) were examined at × 1280 under Zeiss phase contrast and Köhler illumination; this sharpens the contrast of the chromosomes and the grains in the emulsion, and facilitates the detection of unstained cytoplasm as well as of irregularities in the albumin. Under phase contrast the stained bands shift colour towards the violet-blue, without alteration of the banding pattern. By means of an ocular scale free of parallax error the grains in the emulsion within 0.5 μ of the edge of the chromosomes were scored; under the resolution conditions for ^{35}S in squashes (1—1.5 μ) this ensures a recovery of most of the grains associated with the chromosomes[1], and minimizes the contribution from cytoplasm. A per-

[1] As measured in two chromosomes with no cytoplasm nearby (2 R and 3 R in cell 57.1, see later), where practically all the associated grains were recovered, the recovery with our procedure is 73 and 71%, respectively.

manent record of the cytology of each chromosome, of the nearby cytoplasm, and their associated grains, often recognizable as ARGs of bands or interbands, was plotted on scaled paper; thus in the records the overall activity of the chromosome could be read separately from or jointly with the ARGs of bands. Other cell components were studied under the same conditions, their grains counted within 0.5 μ outside their boundaries, and their surfaces measured with an ocular graticule.

The main source of error may have been the activity of the cytoplasm, not infrequently greater than that of the chromosomes: it is quite impossible to visualize small patches of cytoplasm above chromosomes. ["Cytoplasm" also includes nuclear sap, nuclear filaments (EMMENS 1937) and luminar contents, between which it is impractical to differentiate in squash preparations.] An occasional separation between chromosomes and contacting cytoplasm observable in routine squashes (due to contraction during fixation, etc.) appears perhaps more frequently in the squashes treated as here; measurements of the width of chromosomes indicate that this is due to drying just before exposure. It should favour the underestimation of the error due to patches of cytoplasm above chromosomes and, conversely, diminish the contribution from nearby cytoplasm. The intrinsic background of the emulsion was less than one grain per 100 μ^2 and was neglected.

For the evaluation of results certain considerations were necessary. In squashes treated as here the fine detail of chromosomes can never be as good as in routine ones: apart from the procedure itself, the chromosomes have to be observed under a stained and grain-loaded emulsion. As well as cytological clarity, criteria such as straightness for plotting, and absence of overlaps and nearby cytoplasm decided the choice of chromosomes, and made impossible the scoring of complete cells. Few chromosomes meet these criteria in all their parts. It is admitted that some degree of selection was exercised as to activity of the chromosomes, in the sense that, other conditions being equal, the most active chromosome was studied.

ARGs of bands and interbands, though frequently well resolved, were with few exceptions scored only as the full subdivision in which they are contained in BRIDGES' chart (1935). Scores of bands-interbands *per se* would be unreliable unless one is prepared to sacrifice the scoring of full chromosomes to the scoring of only well stretched divisions. The chromocentral (heterochromatic) regions of chromosome 2, as given in the revised chart (BRIDGES 1939, 1942), were not scored; the corresponding regions in the other chromosomes, except the first part of division 20 in chromosome X, were not included in the original chart. This first part of division 20 was included in the plots but was ineffectually scored because of its frequent fusion with the chromocenter. For total activity the less prominent sections of chromosomes were scored by divisions or groups of them. In comparisons between activity and length of chromosomes the well determined lax length (BRIDGES 1942) was taken as the normal parameter: this eliminates having to deal with length dependent on the degree of squashing.

The strain used is an inbred strain of wild type phenotype, except for white eyes and a slight branching of the posterior crossvein. It is free from chromosomal inversions. No chromosomal mutations were present in larvae from the radioactive culture sampled on day 21, or from the 13th generation subcultured in normal food. Of 158 flies hatched from the radioactive culture and of 11,586 flies from twenty-two subsequent generations on normal food, none presented any gross mutant character. The dose in reps per day received by the larvae in the radioactive food was: 8.6 at zero day, 7.5 at day 17 (birth of subcultured flies), and 6.7 at day 32 (birth of last scored flies). These low dosages are not expected to induce mutations in any large scale. As corroborated by the normal aspect of the chromosomes previously mentioned, and the normal development of the larvae, the dosage was harmless.

Results

Activity of the chromosomes

The activity of divisions, or groups of them, has been plotted in Figs. 8—13 for six representatives of each chromosome, and for seven in the case of 4R. No effort was made to study 4L since, due to its small size, it can hardly be detected (Slizynski 1944). The basal portion of chromosome 2L was, in all the samples selected, of unsuitable cytology due to fusions of the chromosome with itself or with the chromocenter.

The activity has been additively plotted so as to analyse the trend of activity per division, or group of them, and the overall activity of each chromosome. Some regions were not scored owing to possible cytoplasmic contamination or to cytological difficulties: the trend

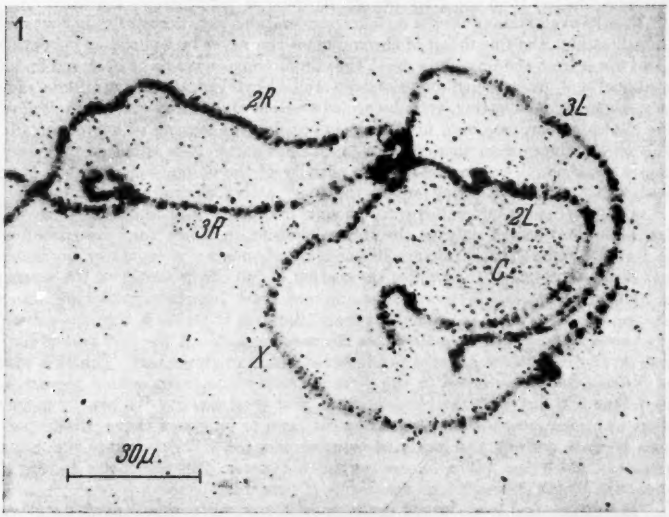
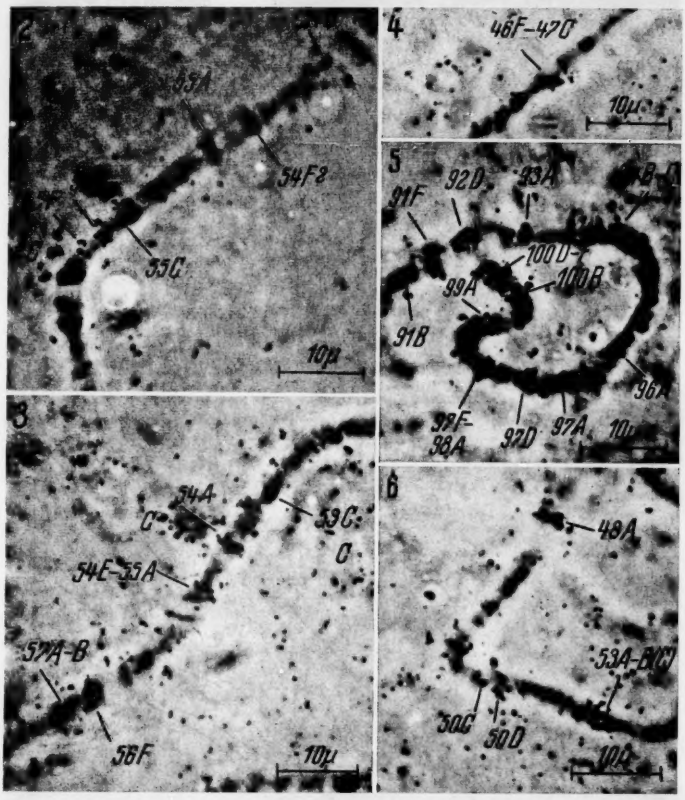


Fig. 1. Chromosomes of cell 57.1 and autoradiograph. Cytoplasm (*C*) is present

cannot be read in divisions comprising such regions, but their missing contribution to the overall activity can be approximately assessed from the scores of the same division in other chromosomes. Regions where scores were suspected of cytoplasmic contamination have been marked. With these reserves in mind, an average maximum activity was subjectively fitted to each chromosome. The length and band density of the chromosomes was unknown at the time this was done.

It can be seen that the trend of all chromosomes in Figs. 8—13 is remarkably constant along their entire length. Neither the proximal nor the distal ends show any differential pattern. Also the curves are



Figs. 2—6. Phase contrast and autoradiographs. Not all photographic grains can be focussed simultaneously with the chromosomes. Cytoplasm: *C*. Figs. 2, 3, 4 and 6: parts of chromosome 2 R. Fig. 5: distal end of chromosome 3 R

independent on the whole of certain cytological characters to be mentioned in a later section. Being uniform, the curves do not correspond with the distribution of heterochromatin depicted by HANNAH (1951), though the scale of plotting is not too appropriate for the comparison.

For a character of this kind, the samples of the second chromosome (Figs. 9 and 10) form a homogeneous group. The same is true of the

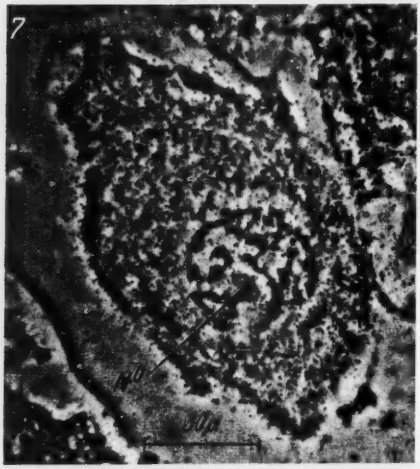


Fig. 7

Fig. 7. Unruptured salivary gland cell and autoradiograph showing less activity in the nuclear sap than in the chromosomes or cytoplasm. The nucleolus ($N°$) is visible

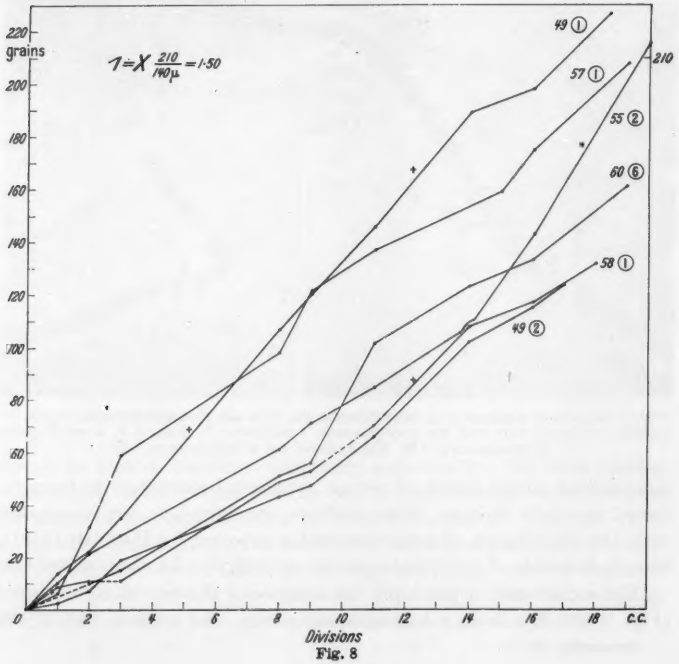


Fig. 8

Fig. 8. Cumulative activity by divisions in chromosome X. Each curve represents one sample of the chromosome. References: cc chromocentral end; + cytoplasm nearby; --- unscored parts. On right ordinate the average maximum total activity. Ratio of the latter and lax length indicated

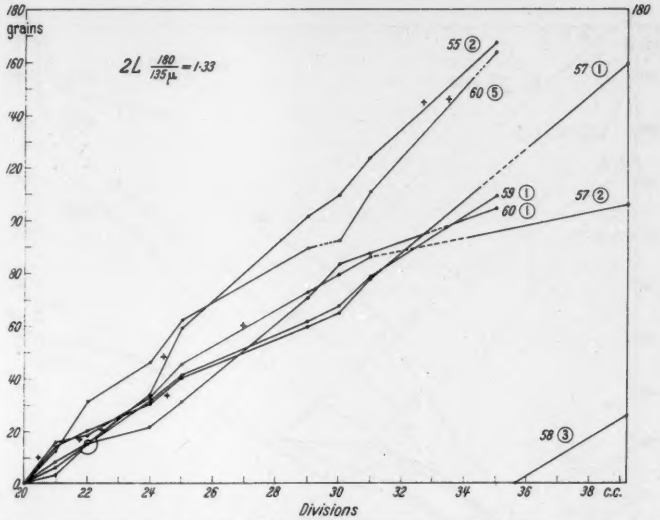


Fig. 9. Cumulative activity by divisions in chromosome 2 L. References as for Fig. 8

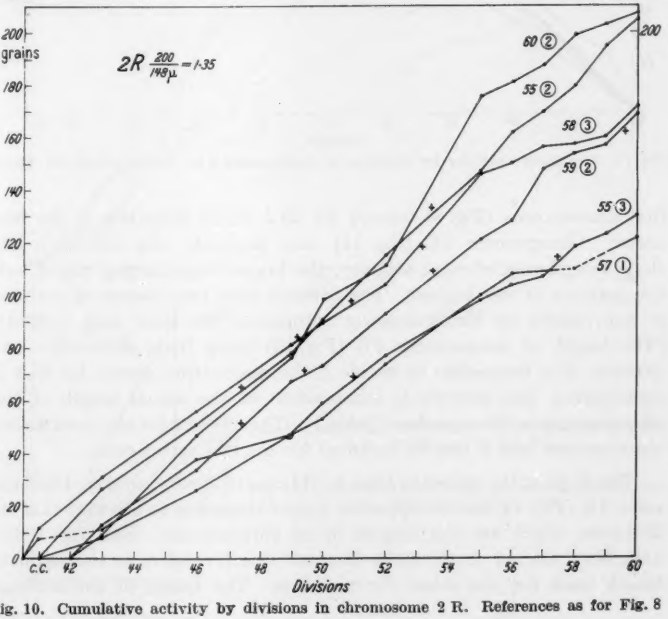


Fig. 10. Cumulative activity by divisions in chromosome 2 R. References as for Fig. 8

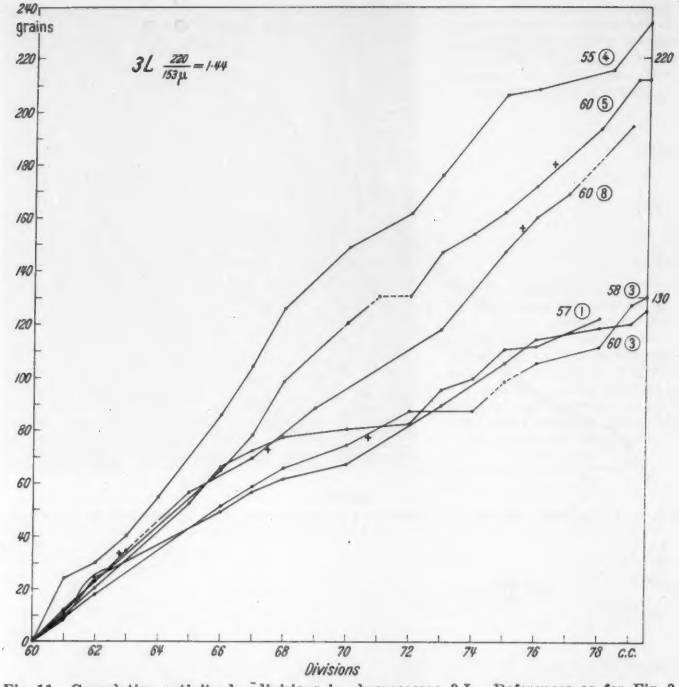


Fig. 11. Cumulative activity by divisions in chromosome 3 L. References as for Fig. 8

first chromosome (Fig. 8), except for 49.2 which definitely is the less active. Chromosome 3L (Fig. 11), and probably also 3R (Fig. 12), show two classes of total activity, the lowest class having about half the activity of the highest. The division into two classes of activity is not caused by contaminating cytoplasm (see later and Table 1). [The length of chromosome 4R (Fig. 13) being little more than one division, it is impossible to decide if the discordance shown by 58.2 is significant.] The activity is independent of the actual length of the chromosomes in the squashes (Table 1). This is clear for the most active chromosomes and it can be assumed for the less active ones.

The slope of the curves in Figs. 8—11 and 13 is very similar. Chromosome 3R (Fig. 12) has an apparent higher slope due to the fact that its divisions, which are the longest of all chromosomes (BRIDGES 1935), have been plotted on the same abscissal length relative to the ordinate length used for the other chromosomes. The ratios of the average

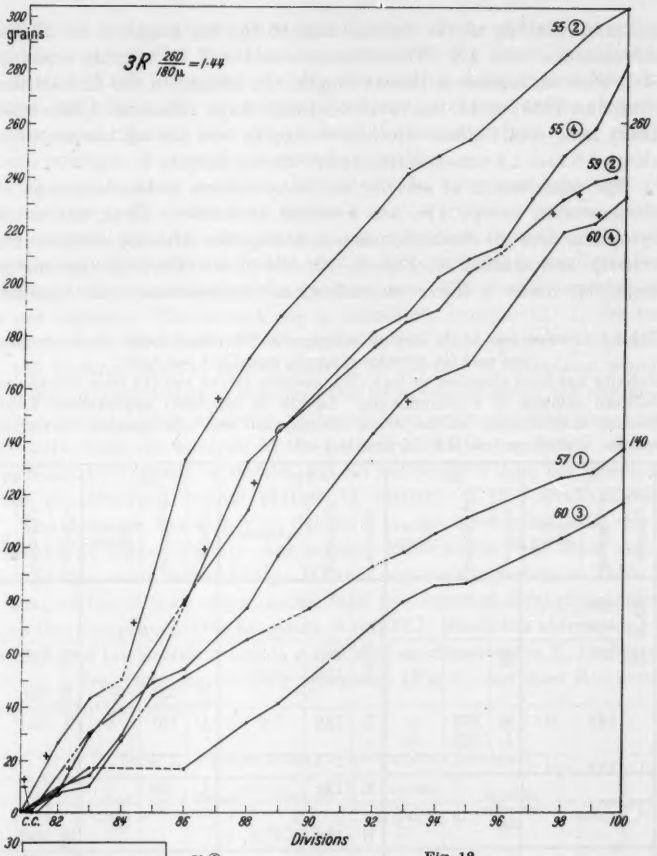


Fig. 12

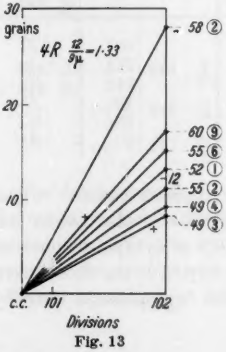


Fig. 13

Fig. 12. Cumulative activity by divisions in chromosome 3 R. References as for Fig. 8

Fig. 13. Cumulative activity by divisions in chromosome 4 R. References as for Fig. 8

maximum activity of the chromosomes to the lax length is for all the chromosomes near 1.4. These ratios would still be roughly equal to each other if, instead of the lax length, the lengths in the first edition (BRIDGES 1935) or in the revised edition maps (BRIDGES 1938, 1939, 1942) were used; these alternative lengths are for all chromosomes about 1.5 and 2.8 times, respectively, the lax length.

The contribution of activity in the cytoplasm to the counts on all chromosomes, except 4R, was assessed as follows. First, the active cytoplasm near the chromosomes, scored together with the chromosomal activity and marked in Figs. 8—12, shows no effect on the curves. Secondly, under a lower magnification chromosomes were classified

Table 1. *Comparison of the level of activity with the actual length of chromosomes and with the presence of nearby cytoplasm* (see text)
Activity has been classified as high (H), medium (M) or low (L) from comparison between samples of a chromosome. Length in brackets: approximate length because measurement of the whole chromosome was not possible. Error not serious. Cytoplasm (see text): 0 to +++

Larvae	Cells	X			2L			2R			3L			3R		
		activity	length (μ)	cyto-plasm	activity	length (μ)	cyto-plasm	activity	length (μ)	cytoplasm	activity	length (μ)	cyto-plasm	activity	length (μ)	cytoplasm
49	1	H	(169)	++												
	2	L	164	++												
55	2	H	217	++	H	90	+++	H	166	++				H	232	++/+++
	3							L	158	+/++						
	4										H	155	0/+	H	246	0/+
57	1		145	0	M	133	+	L	119	0	L	131	0	L	154	0
	2				L	125	+									
58	1	L	133	+/++												
	3							M	134	+/++	L	126	+			
59	1				L	139	+									
	2							H	196	++/+++				H	243	++/+++
60	1				L	126	0/+	H	181	++/+++						
	2							H	267	0						
	3										L	146	+	L	137	+/++
	4													M	218	++/+++
	5				H	(217)	+				H	219	+/++			
	6	H	129	+												
	8										H	145	+/++			

according to the amount and activity of cytoplasm present within about 15 μ outside the chromosome, their length being also taken into consideration, as shown in Table 1. The activity of cytoplasm parallels the activity of the chromosomes in a general way, i. e. the most active chromosomes are associated with the greatest cytoplasmic activity.

But this is not so in every particular instance. Some of the most active chromosomes of Figs. 8—12 are associated with the lowest cytoplasmic activities (Table 1), while typically least active chromosomes may be associated with active cytoplasm. Both most and least active chromosomes occur together with cytoplasm of the lowest activity. The maximum averages of activity are therefore justified.

The relationship between the activity in cytoplasm and that of chromosomes, if tenable, may receive any interpretation between these two extreme ones: (a) chromosomes and cytoplasm from the same cell have a correlated activity, (b) the relationship originates in small patches of cytoplasm contaminating the chromos mes. The first interpretation is not unlikely. The second one is lessolikely because of: 1. the far from constant relationship, 2. the selection practised against cytoplasm, 3. the uniform trend of overall activity in the face of cytoplasm which is rarely uniform either in activity or in its extension along the chromosomes, and 4. other evidence gathered from split chromosomes and, specially, from the analysis of the pattern of ARGs (see later). Some contamination cannot be denied, but on the whole it does not interfere with the observed overall pattern of activity of the chromosomes.

The data are too scanty to decide if in one larva some cells show consistently higher activity over several chromosomes than other cells, though this seems rather likely. What is reasonably obvious in Table 1 is that within cells all chromosomes tend to a common level of activity, with two exceptions. One exception is cell 57.1, which has chromosomes of high and low activity: this is a real case as chromosome X, the most active, is free from surrounding cytoplasm (Fig. 1), nor does it appear larger than the others.

Table 2. *Specific activity of chromosomes* (see text)

	Lax length (μ) A	Average width (μ) B	Surface (μ^2) A × B = C	Average maximum activity D	Specific activity B/A	Average (grains/μ^2)
X	140		350	210	0.60	
2L	135		337	180	0.53	
2R	148	2.5	370	200	0.54	0.56
3L	153		382	220	0.58	
3R	180		450	260	0.58	
4R	9		23	12	0.52	

For later comparisons of activity with other nuclear components, the activity of chromosomes per unit surface (specific activity) is given in Table 2. For the length, the lax length (BRIDGES) was used; in general, the moderately stretched length of the samples is not very different from the lax length. For the width, an average of 2.5 μ was

measured in the squashes (in properly stretched chromosomes, the width varies with varying stretching proportionately much less than the length). This estimate of specific activity can only be an approximation, but it shows a similarity for all the chromosomes, with an average of 0.56 grains/μ^2. This similarity is of course that of the ratios in Figs. 8—13 differently stated.

Activity of bands and interbands

The activity of bands and interbands has been plotted in Figs. 14—19 for the subdivisions containing them. Strong ARGs corresponding to single bands are well resolved and frequent (Figs. 2—6). Broad interbands also show ARGs, but the possibility of light bands being masked by the silver grains is always present: in interbands there is an average of ten submicroscopic bands (< 1,000 Å) per micron (LOWMAN 1956). Compacted sequences of bands and interbands are beyond the resolution of the technique and many of these give ARGs which are plotted as continuous over several subdivisions. Seven samples of each chromosome are plotted, but owing to cytoplasm or to cytological difficulties some regions, i. e. the proximal ends of 2L and 2R, are ineffectually plotted.

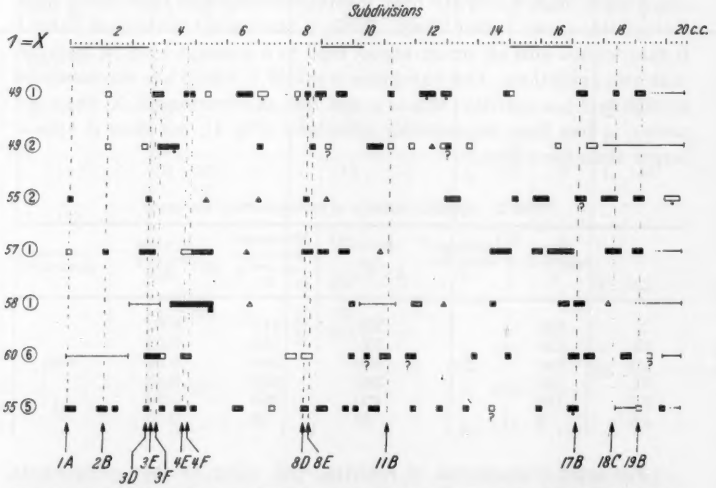


Fig. 14. Autoradiographs by subdivisions in chromosome X. Each horizontal plot represents one sample of the chromosome. References: *cc* chromocentral end; ■ strong ARG; ■ ARG; □ doubtful ARG; △ ARG of any strength in regions of difficult cytology; ? cytological score doubtful; . cytoplasm or another chromosome near ARGs; --- unscored parts; —— (under scale) regions of confident cytological score. Subdivisions showing ARGs in 50 % or more of the samples indicated at the bottom

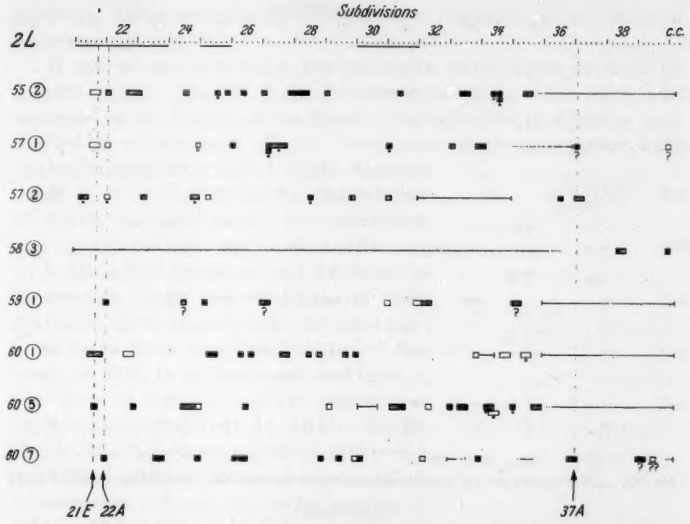


Fig. 15. Autoradiographs by subdivisions in chromosome 2 L. References as for Fig. 14

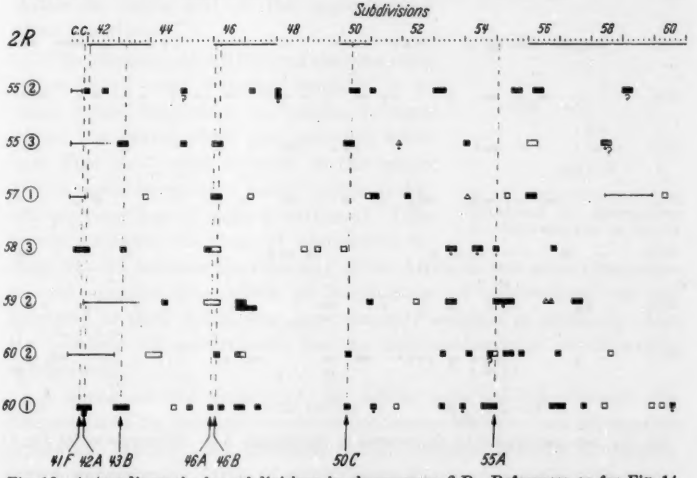


Fig. 16. Autoradiographs by subdivisions in chromosome 2 R. References as for Fig. 14

All but the last chromosome samples plotted in Figs. 14—18, and all samples in Fig. 19, have been previously plotted for overall activity

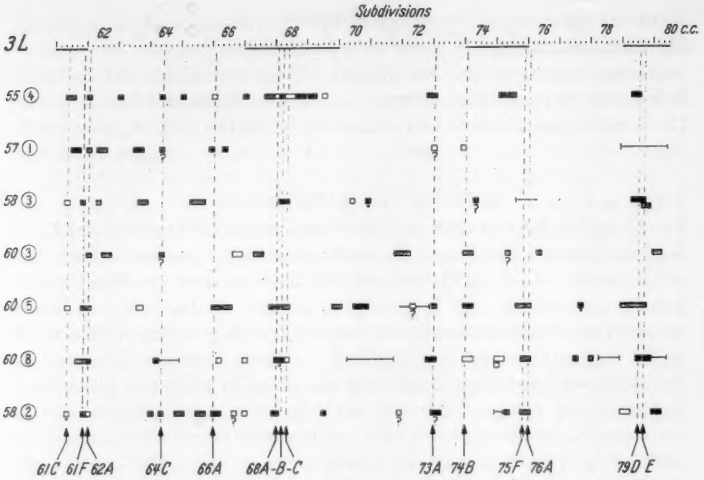


Fig. 17. Autoradiographs by subdivisions in chromosome 3 L. References as for Fig. 14

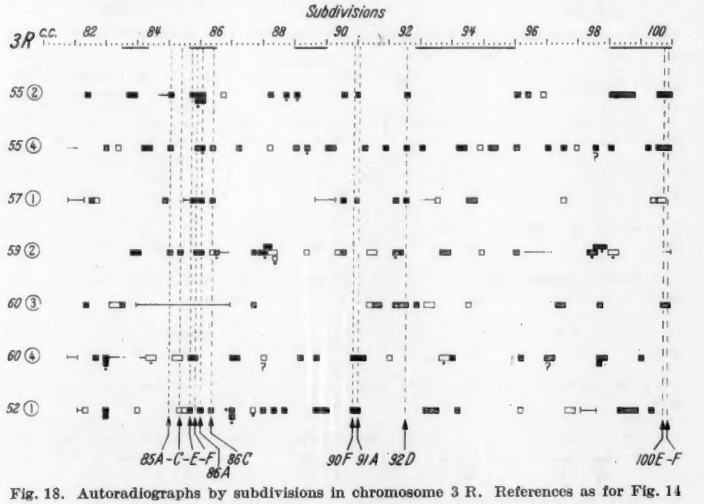


Fig. 18. Autoradiographs by subdivisions in chromosome 3 R. References as for Fig. 14

(Figs. 8—13). In the new chromosome samples not included in Figs. 8—12 only the ARGs of bands and interbands were recorded, and not the remnant of the activity. The subdivisions where cytoplasm may have

produced ARGs over the chromosomes are indicated, as are those of uncertain cytology.

It can be seen that some, but not many, subdivisions show ARGs in over half or more of the subdivisions sampled; these have been indicated at the bottom of the figures. Repeatability of ARGs is most marked in chromosome 2R and both arms of chromosome 3. Very easily distinguishable initial bands of certain subdivisions were examined for the presence of ARGs in cases where the subdivision was clearly readable and showed ARGs: in 15 A, the initial bands showed ARGs in all three cases where the subdivision showed ARGs; in 50 C, in three out of four cases; in 62 A, in three (one doubtful) out of five cases; in 85 F, in all four cases; and in 96 A, in two out of three cases. Other subdivisions are consistently devoid of ARGs, though almost always they are associated with grains not recognisable as ARGs of bands. Of all chromosomes 2 R has the lowest number of ARGs. There is no more repeatability of ARGs in either end of the chromosomes than elsewhere.

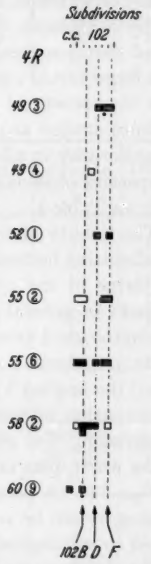


Fig. 19. Autoradiographs by subdivisions in chromosome 4 R. References as for Fig. 14

The presence of ARGs, and therefore the repeatability over different samples, is to some extent dependent on chance because of the few grains which compose each ARG (see Figs. 3—7) (the activity of the whole chromosome being of a larger order is for our purpose free of such a variance). This partly explains the lack of correlation in Figs. 14—18 between the intensity of the ARGs in one subdivision over several samples (the effect of compaction of subdivisions on the intensity of their ARGs was approximately weighed at plotting). Also the plotting by subdivisions has an anti-randomizing effect within subdivisions.

A check on the 'reality of the ARGs' was possible through the occurrence in the preparations of chromosomes with the two chromatids separated. Sixteen split divisions, in four different samples of chromosomes, gave sixteen ARGs of subdivisions in one chromatid with corresponding ARGs on the same subdivision in the sister chromatid (total: 32 ARGs), nine ARGs in one chromatid without a corresponding one in the other, and two ARGs, one in each chromatid, whose correspondence

was dubious. One of the splits, in this case with no ARGs in either chromatid, is shown in Fig. 1. Single chromatids contain half as much protein as double chromatids (Schultz *et al.* 1955). Considering then that the lesser activity in chromatids than in chromosomes increases the variance of the ARGs, these figures mean a good degree of correspondence. Split chromatids in which both of them are clearly readable are not often found, and the present ones were not selected at all against nearby cytoplasm: its abundance resembled that found in most of the scored chromosomes. On the whole the ARGs of bands-interbands are then largely 'real', as was to be expected from the close correspondence with the structural detail of the chromosomes (Figs. 2—6). The best evidence against any serious contamination by cytoplasm is the statistical uniformity in all samples of the activity as ARGs (see next section), irrespective of their classification as to the presence of nearby cytoplasm given in Table 1.

The 'reality of the pattern of ARGs' can be assessed less efficiently. Randomizing factors are: variance of ARGs, cytoplasmic contamination, and error in the cytological score. The first two have already been treated: in general they will probably have a not too serious effect. The cytological error would be as follows. For divisions underlined in the map scales at top of Figs. 14—18 and for subdivisions bordering on them, the scoring by subdivisions is generally accurate; with the slight condensation tolerated in some samples it can be to plus or minus one subdivision. For other divisions, the score will often be accurate to, at the worst, plus or minus 3 subdivisions. From the overall appearance of Figs. 14—18, particularly from the intercalation of regions of accurate scoring, it can be concluded that the pattern of ARGs cannot be attributed to cytological error: the regions of confident scoring are no different, either in uniformity or in variability, from the others. As this is independent of the class of activity of the chromosomes, and as chromosomes strictly comparable in respect of class of activity and presence of nearby cytoplasm (Table 1), tend to agree where the others agree, all samples were plotted jointly in Figs. 14—19.

Relation between ARGs of bands and interbands and the activity of the entire chromosome

The clustering of ARGs in certain regions of the chromosome (Figs. 14—18) does not have any effect on the trend of the overall activity (Figs. 8—12) of these regions. Exceptions are: division 9 in sample 57.1; divisions 10 and 11 in 60.6; divisions 53 to 55 in 60.2. Conversely, the trend of the curve is the same in regions where no ARGs can be recognized, or in whole chromosomes with fewer ARGs than the others (i.e. 2R), as it is in regions where ARGs are present.

Table 3. *Subdivisions showing ARGs as per thousand of total activity*
Data from regions scored in Figs. 14—19

X Cells	Total activity (grains) A	Subdivisions with ARGs B	1000 × B / A
49.1	227	30	132
49.2	124	21	169
55.2	216	26	120
57.1	208	34	163
58.1	132	19	144
60.6	161	25	155
		Average	147

2L Cells	Total activity (grains) A	Subdivisions with ARGs B	1000 × B / A
55.2	167	23	138
57.1	160	17	106
57.2	106	14	132
59.1	109	12	110
60.1	104	20	192
60.5	164	22	134
		Average	135

2R Cells	Total activity (grains) A	Subdivisions with ARGs B	1000 × B / A
55.2	205	18	88
55.3	130	13	100
57.1	122	13	107
58.3	172	16	93
59.2	169	20	118
60.1	260	30	115
60.2	207	16	77
		Average	100

3L Cells	Total activity (grains) A	Subdivisions with ARGs B	1000 × B / A
55.4	234	27	115
57.1	122	14	115
58.3	129	18	140
60.3	125	17	136
60.5	212	31	146
60.8	195	22	113
		Average	128

3R Cells	Total activity (grains) A	Subdivisions with ARGs B	1000 × B / A
55.2	304	25	82
55.4	282	35	124
57.1	137	18	131
59.2	240	30	125
60.3	116	20	172
60.4	232	26	112
		Average	124

4R Cells	Total activity (grains) A	Subdivisions with ARGs B	1000 × B / A
49.3	8	3	375
49.4	9	1	111
52.1	13	2	154
55.2	11	4	364
55.6	15	5	333
60.9	17	2	118

The number of ARGs in a chromosome, as can be read from Figs. 14—18, is related to the overall activity of the chromosome. In Table 3 the number of subdivisions with ARGs in each chromosome has been calculated as the per thousand of the total activity of that chromosome. Even without statistical treatment, which could only be artificial here on account of the crudeness of the parameter 'subdivisions with ARGs' and the lack of scores in parts, these values reasonably prove that the number of ARGs in a chromosome bears a relation to the total activity of that chromosome. For example, the three samples of chromosome 3L (cells 57.1, 58.3 and 60.3) and the two of 3R (cells 57.1 and 60.3), belonging to the lower class of activity, are within the range of values

for the other samples of the same chromosomes. Chromosome 2 R shows the lowest values: the average value is 27% less than the combined averages of the other chromosomes, 4 R excluded. Values for chromosome 4 R are the most variable due to the small figures entering the calculation. The actual length of the chromosomes as given in Table 1 does not determine the number of ARGs. This, which as mentioned later applies also to the percentage of activity as ARGs, indicates that the stretching was adequate enough to prevent ARGs arising from the compaction of bands and interbands of low activity.

Table 4. *Activity and density of band numbers. Percentage of activity as ARGs*

	X		2L		2R		3L		3R		4R	
Number of photographic grains per μ of chromosome (A) . .	1.50		1.33		1.35		1.44		1.44		1.33	
Relative density of band numbers (B) .	1.00		0.83		1.06		0.80		0.91		*	
A/B	1.50		1.60		1.27		1.80		1.58			
	cell	%	cell	%	cell	%	cell	%	cell	%	cell	%
Percentage of total activity recognizable as ARGs	49.1	52	55.2	46	55.2	40	55.4	49	55.2	39	49.3	63
	49.2	50	57.1	40	55.3	32	57.1	39	55.4	54	49.4	22
	55.2	46	57.2	46	57.1	37	58.3	57	57.1	48	52.1	85
	57.1	54	59.1	40	58.3	47	60.3	44	59.2	62	55.2	66
	58.1	45	60.1	58	59.2	42	60.5	55	60.3	58	55.6	73
	60.6	60	60.5	54	60.1	48	60.8	52	60.4	57	60.9	65
					60.2	36						
Mean $\pm$ standard error	51.1 $\pm$2.2		47.3 $\pm$2.9		40.3 $\pm$2.2		49.3 $\pm$2.7		53.0 $\pm$3.3		62.3 $\pm$8.7	
χ^2 .	2.98		5.69		5.09		4.72		6.49		36.50	
Probability (%) . . .	ca 70		$30 < P < 50$		$50 < P < 70$		$30 < P < 50$		$20 < P < 30$		$\ll 0.1$	

* Not given by Bridges (1942).

Probability of differences of the means (%)	Between 2L—2R	$F^1_{11} = 3.71$ $5 < P < 10$
	2R—3L	$F^1_{11} = 6.63$ $1 < P < 5$
	2L—3R	$F^1_{10} = 1.57$ $P > 20$

Table 4 gives the percentage of the total activity of each chromosome that was recognized as ARGs. An extra sample of chromosome 2 R, 60.1, which is very active (total count: 260 grains) and shows the greatest number of ARGs in Fig. 16, has been included. In Table 4 all chromosomes show values near 50%, except 2 R and 4 R. As judged by the χ^2's, each chromosome presents a homogeneous group of percentages, except 4 R. In the latter, this is heterogeneous due to the

small size of the original counts, and has not been used for further analysis. In Table 5 the analysis has been carried further, using the actual counts to weigh the variable size of samples (Table 3, column A). χ^2's inside columns indicate that the ratios of counts in each column are heterogeneous or nearly so between themselves, but this which is a property of the material or the technique does not invalidate further analysis. In a comparison between columns, which weighs the heterogeneities inside columns, all columns if jointly considered are heterogeneous. As canbe seen from the component χ^2's, column 2 R is responsible for this, and its removal makes the rest homogeneous. The same result is obtained if instead of the actual counts the percentages are used. In Table 4, the mean of chromosome 2 R is significantly lower than that of any of the other chromosomes, except 2 L, which do not differ significantly among themselves. However, when count size is weighed as in Table 5, chromosome 2 L resembles all the other chromosomes to the

Table 5. *Analysis of variance in the actual counts giving the percentages in Table 4*

Chromosomes	Inside columns			Between columns					
	Degrees of freedom	χ^2	Probability (%)	Components of x_1^2	$F_{\frac{1}{20}}^{4}$	Probability (%)	Components of x_2^2	$F_{\frac{1}{20}}^{1}$	Probability (%)
2R	6	14.44	2<P<5	25.70					
X	5	9.20	5<P<10	4.09			0.38		
2L	5	12.86	2<P<5	0.42	2.99	1<*P*<5	3.51	0.54	≫20
3L	5	12.80	2<P<5	0.85			0.19		
3R	5	35.90	≪0.1	8.15			1.69		
4R	5	9.89	5<P<10						
	31	95.09	≪0-1						

exclusion of 2 R. Thus, chromosome 2 R has the lowest percentage of total activity as ARGs: the percentage is 20% less than in the other chromosomes. No artifact could be envisaged as producing this lower percentage. Its actual length bore the same relation to the lax length as the other chromosomes in our samples. This analysis disproves any serious interference by cytoplasm.

Table 6 gives the mean intensity of ARGs. The intensity of ARGs in an extensible object such as chromosomes is not a reliable index for comparisons. To allow for the extension of the ARGs most of them were computed as for integers from one to several subdivisions, a few as for integers plus 0.5. Each of two ARGs in a single subdivision was computed as in a subdivision. The last two treatments avoid blowing the figures as would result from division by less than unity, and respect as much as possible the individuality of each ARG; these cases were not many in Figs. 14—18. As number of subdivisions enters

Table 6. *Number of photographic grains per autoradiograph per subdivision* (see text)

X Cell	Grains per ARG per subdivision	2L Cell	Grains per ARG per subdivision	2R Cell	Grains per ARG per subdivision
49.1	4.29 ± 0.39*	55.2	4.01 ± 0.43	55.2	4.99 ± 0.65
49.2	3.48 ± 0.34	57.1	4.48 ± 0.69	55.3	4.00 ± 0.50
55.2	4.69 ± 0.49	57.2	4.33 ± 0.59	57.1	3.55 ± 0.45
55.5	4.74 ± 0.39	59.1	4.56 ± 0.70	58.3	5.22 ± 0.63
57.1	3.86 ± 0.35	60.1	3.86 ± 0.47	59.2	4.38 ± 0.50
58.1	3.79 ± 0.40	60.5	4.53 ± 0.48	60.1	4.13 ± 0.34
60.6	4.32 ± 0.47	60.7	4.93 ± 0.50	60.2	5.61 ± 0.54
Common mean	4.16 ± 0.17	Common mean	4.38 ± 0.13	Common mean	4.55 ± 0.27

3L Cell	Grains per ARG per subdivision	3R Cell	Grains per ARG per subdivision
55.4	5.26 ± 0.62	52.1	4.19 ± 0.38
57.1	4.16 ± 0.50	55.2	5.26 ± 0.68
58.2	4.37 ± 0.44	55.4	5.43 ± 0.45
58.3	4.77 ± 0.58	57.1	4.21 ± 0.49
60.3	4.37 ± 0.90	59.2	5.03 ± 0.44
60.5	4.55 ± 0.55	60.3	3.36 ± 0.34
60.8	4.56 ± 0.65	60.4	5.47 ± 0.63
Common mean	4.57 ± 0.13	Common mean	4.70 ± 0.30

* Standard errors

the calculations in Table 6, the values are not suitable for analysis. The means of samples were considered as independent observations of equal weight and a common mean and standard error were obtained. It is evident that they do not differ much. The values for chromosome 2 R are no lower than those for the other chromosome.

On the upper part of Table 4 the activity per micron of chromosome has been divided by the relative numbers of bands per micron (BRIDGES 1942, Table 3, col. 4). Similar ratios result if the relative numbers with double bands counted as one (*loc. cit.*, col. 5) are used. It can be seen that the lowest ratio corresponds to 2 R. The lowest values will also correspond to 2 R for the following ratios: total activity over density of band numbers, or, activity per micron over total number of bands. The lowest value for 2 R means that this chromosome shows in a statistical sense less activity per single band and/or interband than the other chromosomes.

From Table 1, the percentages of ARGs as given in Table 4 are largely independent of the classes of activity in which the chromosomes belong, of the actual length of the chromosomes, and of the presence

of nearby cytoplasm. Thus, the number of ARGs (see before), but not the percentage of activity as ARGs, depends on the level of activity of each chromosome.

Activity of certain parts of chromosomes

In *Drosophila melanogaster* the chromocenter is formed by the coalescent centromeres; it comprises the heterochromatic regions which, with the exception of division 20, have been left out of the plots. All chromosomes are metacentric with the exception of chromosome X which is telocentric. Table 7 gives the specific activity of ten chromocenters suitable for scoring, possibly incorporating 4 L but, at least most of them, not 4 R. The last chromocenter listed was the only one recognisably overstretched and was not included in the average. It is almost certain that differences in the ratios are partly due to squashing. The chromocenters have DNA and look optically quite dense. As judged by the fine adjustment of the microscope they were as a whole not appreciably thicker than the rest of the preparation, but internal variations of thickness are most certain. Considering this source of error and the small dimensions of the object the ratios are reasonably consistent. Most frequently, however, chromocenters will impress the observer as being much more active than those scored, owing to condensation on them of basal parts of the chromosomes.

Table 7. *Specific activity of chromocenters* (see text)

Cell	Surface (μ^2) A	Grains B	Specific activity B/A	Average (grains/μ^2)
55.2	35.8	28	0.78	
55.3	56.3	31	0.55	
55.7	61.4	25	0.41	
57.1	23.0	18	0.78	
58.2	43.5	26	0.60	0.66
58.3	20.5	13	0.63	
58.5	17.9	15	0.84	
60.1	30.7	23	0.75	
60.1	48.6	28	0.58	
60.11	125.4	30	0.24	

Certain regions of the chromosomes are characterized by extrusion of chromosomal material: these regions are called "nucleolus-like" in the X (regions 5 C, 7 BC, 11 A, 12 EF and 19 EF; BRIDGES 1938, SLIZYNSKI 1945) and "puffs" in other chromosomes (42 A, 47 C; 68 C; 85 F; BRIDGES 1935 and revisions). In Figs. 8—12 and 14—18 these regions are not particularly associated with foci of activity near, or with ARGs in, the chromosomes; a systematic error originating from such a likely source is thus disproved. On the other hand, these subdivisions, which in chromosome X are of heterochromatic nature, are also not particularly dissociated from ARGs. When specially looked for the extrusions were at times recognized as distinct from cytoplasm, but frequently they were detached from the chromosomes and likely

to be taken for cytoplasm of variable, but not high, activity. Region 20 B—D, where the true nucleolus is attached (BRIDGES 1938, KAUFMANN 1938), forms part of the basal heterochromatin of chromosome X and was ineffectually scored.

Observations on other constituents of the salivary gland cell

Nucleoli are generally recognizable in these preparations under phase contrast, and tend to occur near the chromocenter through often they are displaced as a result of the squashing. The nucleoli possess a discrete boundary (though probably not a membrane), a more homogeneous ground substance than the cytoplasm, and other internal structure which is discussed elsewhere (SIRLIN 1958). They appear no thicker than the rest of the preparation. The total activity of nucleoli was assessed as follows: eleven nucleoli favourable for scoring are entered in Table 8. The ratios in the fourth column indicate that the activity per unit surface (specific activity) of the nucleolus is constant. The nucleoli of larvae 55, and perhaps that of 59, are smaller than the other nucleoli. The average specific activity, excluding cell 60.5 with an almost certainly oversquashed nucleolus, is 0.51 grains/μ^2. The specific activity of small nucleoli (cell 55; and 59?), higher as a group than for the other nucleoli, could be due to a lower stretchability when size is small. The distribution of activity inside the nucleolus will be published separately (SIRLIN 1958).

Table 8. *Specific activity of nucleoli and comparison with activity of cytoplasm* (see text)

Cell	Total number of photographic grains A	Surface (μ^2) B	Specific activity A/B	Average (grains/μ^2)	Activity per unit surface of nucleolus compared with cytoplasm of same cell
49.2	133	331	0.40		less
55.1	87	162	0.54		less
55.2	79	147	0.54		equal
55.4	87	147	0.59		equal or higher
55.5	85	148	0.57		equal
57.2	152	440	0.35	0.51	equal
58.1	259	440	0.59		less
59.1	100	165	0.61		higher
60.1	219	412	0.53		higher
60.4	147	370	0.40		equal
60.5	176	605	0.29		higher

Cytoplasm in these preparations forms a discontinuous layer of variable activity, probably due to uneven stretching (chromosomes and nucleoli are certainly much less deformable). The activity per unit surface becomes then unreliable for quantitation. In Table 8 this is proved by a subjective comparison made for each cell of the activities of the nucleolus and those of the most active cytoplasm. The total activity of the cytoplasm is far greater than that of the whole nucleus. The activity of the nuclear sap is low compared with that of the other nuclear components. These last two points are illustrated in an un-

ruptured cell in Fig. 7. This is not a fully grown, distal cell, but cells of the latter type show about the same picture.

Certain roundish, most likely secretory, granules in the cytoplasm, measuring from a few up to 18 μ diameter according to the squashing, are very active. They were clearly observed in larvae 52, 55 and 60. Larvae 57 and 58 showed granules which were less dense, more stretchable and less active than those in the other larvae; in other larvae this type of granule was seen to approach the appearance of the denser ones by a series of intermediate steps. In larvae 49 and 59 none of these granules were seen.

General discussion

The biochemical background: nuclear proteins

Proteins are the main components of the nucleus and they can amount up to 80% of its dry weight (ALLFREY, MIRSKY and STERN 1955). Their abundance in the nucleus, unlike that of DNA, varies concurrently with the physiological status of the cell: this has been particularly shown for residual protein of the chromosome (MIRSKY and RIS 1951). Chemical analyses of protein in interphase chromosomes, isolated from various animals, show that histones and residual proteins are the major fractions. Whole nuclei contain little albumin (cf. DOUNCE 1955) and abundant globulin (KIRKHAM and THOMAS 1953) which, however, has not been reported for isolated chromosomes, from which it could have been lost during isolation. Histones are a complex of proteins (STEDMAN *et al.*, 1954, 1957; DALY and MIRSKY 1955) and they are the sole basic protein in somatic nuclei, the other basic proteins, protamins, occurring only in sperm-heads, though not in all types of them. The chromosomal content in histones or residual protein is variable, according to the source tissue and the preparative technique, but any of them can reach 40% or more. Residual protein of chromosomes (chromosomin, STEDMAN and STEDMAN 1943; MIRSKY and RIS 1947) is probably the same as an acidic lipoprotein obtained from isolated nuclei and chromosomes (cf. WANG, MAYER and THOMAS 1953); the matter is accompanied by a small fraction differing from it in its solubility. In combination with DNA it is responsible for the structural integrity of the chromosome (MIRSKY and RIS 1951). Up to about 10% RNA is attached to residual protein, the proportion varying with tissues; some of it is attached to histones. DNA, making up to 40% of the dry weight of the isolated chromosomes, is in part attached to residual protein and in part through salt-linkage to histone (MIRSKY and RIS 1951). For nuclei of different tissues, histones (STEDMAN *et al.* 1954, 1957; DALY, MIRSKY and RIS 1951) and chromosomin (STEDMAN, pers. com.) vary somewhat in composition or physical properties. The

nuclear sap contains a protein complex, but sulphur-aminoacids were not studied (Brown *et al.*, 1950). Little is known of the chemical correlation between nuclear sap and chromosomes.

Chemical analyses of sulphur in chromosome proteins are available. The sulphur in histones plus residual proteins of isolated chromosomes is 1.7% (Claude and Potter 1943). Sulphur-aminoacids were not detected in chromatograms of hydrolysates of isolated chromosomes, though part of them may have been destroyed during hydrolysis (Blumel and Kirby 1948; Yasuzumi and Miyao 1951). Davison *et al.* (1954) summarized the conflicting evidence on the content of sulphur-aminoacids in histones: they possibly contain no cystine and some fractions, no methionine. The bulk of some histones (β and γ histones) contains about 1.5% methionine and no cyst(e)ine (Stedman *et al.* 1957 and pers. com.). Some chromosomins contain about 2% methionine and no cyst(e)ine (Stedman, pers. com.). Other residual proteins contain 0.4% cystine (Stedman 1943) or methionine and cyst(e)ine (Wang *et al.* 1953). In general proteins of high type are richer in sulphur than the aforementioned.

Protein studies of the salivary gland chromosomes of Drosophila *in situ* are also available, but the types of proteins present have not been fully clarified. Non-histone proteins form the backbone of chromosomes (Kaufmann *et al.* 1951; Mirsky and Ris 1947, in *Sciara*; Serra 1947). What amounts to that continuum of residual protein has been observed by Lowman (1956) in the electron microscope as strands of 100 Å width, arranged in a spiral along the polytene chromosome (see also Ambrose 1956). The model proposed is based on the chemical evidence for a more stoichiometrical relationship of histone, than of residual protein, with DNA. Similar observations were more recently made by Yasuzumi (1957). Ris (1957) has proposed an elementary nucleoprotein fibril of 200 Å width as universal for all types of chromosomes. By staining methods the bands appear rich in tyrosine, tryptophan, histidine and NH_2 groups, while the interbands contain only traces of these aminoacids but are rich in NH_2 (Danielli 1947). The presence of tryptophan, which is absent from, or scarce in, histones, indicates the occurrence of proteins of higher type in the bands. It was concluded that the interbands could not be composed of typical histones or of chromosomin (both should contain tyrosine and histidine; and chromosomin should also contain tryptophan); the characteristic aromatic groups of both of these proteins are present almost entirely in bands. But the possibility of the aminoacids in the interbands being in too low concentration to be detected (see later) warns against interpreting qualitatively data of this kind. In *Chironomus* tyrosine was found in bands and interbands, but differences were not stated (Bari-

GOZZI 1937). RNA is linked with an acidic (tryptophan-rich) protein (KAUFMANN *et al.* 1951). Formerly, CASPERSSON determined the concentrations and types of proteins in bands and interbands using their ultraviolet absorption (summarized in CASPERSSON 1950). Later criticism of his technique contended that these results should be regarded more as indicative of differences in density than as ratios of any particular substances (THORELL 1955). More recently, CASPERSSON (1956) maintained the soundness of his original data when considered for total protein. This is higher in bands than in interbands. Residual protein behaves in this way (MCDONOUGH *et al.* 1952). Recently, HORN and WARD (1957) have found correspondence between histones and DNA bands in one species of Drosophila, which is in accord with the early interpretation by CASPERSSON. Generalizing from data gathered from Drosophila as well as other organisms, CASPERSSON indicates a recurring cycle in the relative amount of chromosomal protein with a maximum at interphase and a minimum at metaphase. This was contradicted by RIS (1947) who found the relative amount of total protein, and the ratio of histones to residual proteins, constant during mitosis. In *Chironomus* bands can be 10 times denser than interbands (ENGSTRÖM and RUCH 1951). No unambiguous answer has yet been given to the question of the concentration of non-histone proteins along the chromosomes.

The protein sulphur of chromosomes *in situ* has been reviewed by BRACHET (1950). From colouring reactions in very different types of cells it is difficult to assess its abundance in chromosomes, though some of the completely negative observations are surely due to the limited sensitivity of the reaction. Sea-urchin egg chromosomes are rich in SH (DAN 1957). Chromosome increase their SH and -SS- groups during tissue regeneration (MARSHALL and BARRNETT 1957). In the chromosomes of the early chick embryo variable and, at times, no incorporation of methionine was found (SIRLIN and WADDINGTON 1956). NH_2 and SH groups in the salivary gland chromosomes have been jointly stained (DANIELLI 1949) and though unstated they seem to abound in certain bands. With progressing spermatogenesis the SH groups in the nucleus increase; it could be expected that these groups belong in the chromosomes, which fill more and more of the nuclear volume during spermatogenesis, but this has not been proved. The protein sulphur of the nuclear sap is given in the same review (BRACHET 1950).

The protein chemistry of the nucleolus has been lately reviewed (VINCENT 1955). The total protein can be quite high, up to 90%, and in a state of low hydration. Most of it seems of a high type, albumin-like according to some views. There is also basic protein (HORN and WARD

1957) but perhaps not as much as originally claimed. There is about 5% RNA and some DNA in nucleoli, but to what type of protein they are attached it is not known. No unequivocal description of the non-histone proteins in the nucleolus of Drosophila is available.

Some work on the protein-sulphur content of nucleoli *in situ* has also been reviewed by Brachet (1950). In nucleoli of the early chick embryo the turnover of sulphur proteins is as high as or higher than for any other cell component (Sirlin and Waddington 1956). Nucleoli become richer in SH and -SS- groups during tissue regeneration (Marshall and Barrnett 1957). The ratio SH:SS groups in total proteins of nucleoli of starfish ova has been measured photometrically (Teiger, Farah and di Stefano 1957).

The information given by ARGs as used here

ARGs of intact embryos labelled with methionine-^{35}S detect the tracer as aminoacids incorporated into proteins through peptide linkage (Sirlin, in press). In our material, diversion of the tracer into sulphated esters can be discounted, as it has shown that sulphate-^{35}S is not incorporated into any component of the cells (Pelc 1956). In Drosophila, as in other higher organisms, the sulphur of methionine acts as the source for the sulphur in the other sulphur-aminoacids, cystine-cysteine (Rudkin and Schultz 1947). These three aminoacids are widely distributed among all types of proteins, with the exception of histones which are poor, and protamines which are devoid of them. The D-methionine presented with the tracer is readily invertible by the whole animal into the natural isomer (see Almquist, in Block and Weiss 1956).

The amount of tracer in a living system labelled *ab initio* gives a measure of the total *amount* of tracer incorporated in any chemical fraction. It is not a measure of the *rate* of incorporation (turnover or synthesis) as it is with the labelling of preformed systems over short periods. The measurement of the size of a labelled fraction by the amount of tracer in it is an absolute measurement, i.e. is independent of the time in which different parts of the fraction were constituted, provided that the ratio ^{35}S: ^{32}S in the utilizable sulphur is kept constant. In a complex system, such as a Drosophila culture, the latter is obviously an approximate assumption. Ratios of activity in our material indicate then the final, relative amounts of protein sulphur. On the average the present ARGs were produced by 1.3×10^4 ^{35}S-atoms in the chromosome proteins[1].

[1] Considering yield = 1.8 grains per electron (perpendicular incidence; Lamerton and Harriss 1954), geometry = 50%, and recovery in scores = 72% (see footnote p. 120), a total per chromosome of 200 grains (taken as a typical figure) made deve-

Loss of proteins will have occurred during the procedure, particularly the acid hydrolysis. It has been claimed that acetic acid does not fix albumin (cf. WOLMAN 1955), but such a loss would not be serious as the content in albumin of the nucleus is low. Two hours fixation in alcohol: acetic acid removes 26.5% of the histone of isolated nuclei (STEDMAN and STEDMAN 1950). The acid hydrolysis should extract all or almost all the histones remaining after fixation. In fact, it is one of the standard procedures for removing histone. DI STEFANO (1948) concluded, by indirect estimation of histones in fixed tissue, that N HCl at 60°C removed all histones after 12 to 24 minutes. By direct chemical determinations on isolated nuclei, similarly fixed, histones were totally removed by 10 minutes of hydrolysis (STEDMAN and STEDMAN 1950). Our material was hydrolysed for 5 minutes, as even less than this is the optimum for reading the polytene chromosomes with the stain used (SLIZYNSKA, pers. com.). Most of the histones were probably being removed, to which effect the fixation should also count. The residual protein of the chromosome is more resistant to extraction and is probably not greatly affected by our procedures. On the other hand, it may be that nuclear globulin or any protein of the nuclear sap have been fixed in the chromosomes; fixed chromosomes show no matrix or envelopes (RIS 1957) that may have been missed in the chemical analyses. The interpretations that follow have been made in terms of residual protein as defined before, as this protein is expected to comprise a large part of the polytenic chromosomes, and as it is the only one definitely known to subsist after our procedures. Needless to say, this may be an oversimplification and should a large protein fraction other than residual protein remain in any of the nuclear components sampled, our interpretations would have to be adjusted as in terms of all these proteins jointly.

The interpretation and discussion of results

In these experiments it has been tried to study a uniform batch of larvae. It will be seen that the sensitivity of the method permitted distinction of developmental differences in individual samples of chromosomes. It has been shown that genotype, including sex, and environment, i.e. temperature, can determine to some extent the chemistry

lopable during an exposure of 3 days, started one day after fixation, means 1.32×10^4 ^{35}S-atoms present per chromosome at the time of fixation (with three minutes development of the films as used here the total grain count for the chromosomes should be lower than with a more standard development time, i.e. five minutes). As at that time the labelling in the tracer itself was $1:8.5 \times 10^4$, that figure represents an order of 10^9 protein-sulphur atoms in the chromosome. As the tracer was not the only source of protein sulphur in the food the actual figure should be higher.

of these nuclei (CASPERSSON and SCHULTZ 1940; SCHULTZ *et al.* 1940); of these two variables the first was the most necessary to control. By using genetically homozygous flies the genotype variation is practically eliminated, and the reaction to environment is presumably made uniform. The conclusions apply to the female salivary gland nucleus. Functionally this is to be regarded as an interphase nucleus.

The protein sulphur in the hydrolysed chromosomes

In what follows when we speak of the activity of any chromosome, we refer to the average maximum activity resulting from all samples of that chromosome. In biological work this attitude hardly needs justification. A second class of activity, lower than the average maximum, in chromosome 3 will be separately considered.

From the data we conclude that the concentration of protein sulphur when plotted by divisions is uniform along the entire length (in first approximation = mass) of the chromosome complement. This results from the similarity of the ratio of activity per unit length both within and between chromosomes. The correspondence between amount of protein sulphur and chromosome mass could be checked in males, where the single X chromatid is expected to contain half the amount per unit length of the other two-chromatid chromosomes. In the text the reasons have been given for considering this as a true fact and not as the outcome of systematic error. Plotting the cumulative activity by chromosomal units of length smaller than the divisions in BRIDGES map would not alter the general slope of the curves but would produce more zig-zag-like curves, eventually becoming a plotting of resolved ARGs versus unresolved activity.

The simplest chemical explanation of this situation is that the uniform protein sulphur in the chromosome stands for uniform residual protein. So far, when we say "uniform" we refer to the chromosome at the divisional scale of plotting[1]. It should not be supposed that we are dealing only with the structural framework of the chromosome and not with one substrate of its physiological, ultimately genic, function. Either *in vivo* or *in vitro* residual protein was found the metabolically most active protein of the nucleus (ALLFREY *et al.* 1957; DALY *et al.* 1951; HOBERMAN and PERALTA 1952), more so than histones, and at times it approximates the activity of cytoplasmic proteins. In nuclei it is only

[1] Note added in proofs. — RUDKIN, G. T., and S. L. CORLETTE [Proc. nat. Acad. Sci. U.S.A. **43**, 964—968 (1957)] have determined by photometry that different regions of one chromosome in *Rhynchosciara angelae*, except perhaps the regions with puffs, contain similar amounts of protein after fixation with acetic acid and extraction with hot trichloroacetic acid. This would leave behind residual protein and perhaps some histone.

occasionally second in activity to a small diffusible ribonucleoprotein fraction. Its amount shows a correlation, greater than that of histones, with the amount of active cytoplasm surrounding it; histones seem more stoichiometrically arranged with DNA (cf. ALFERT 1955; KNOBLOCH and VENDRELY 1956). Its activity varies parallel to that of cytoplasm when the overall protein metabolism is made to alter, as does that of histones (ALLFREY *et al.* 1955a). Residual protein is bound in the chromosome with both DNA and RNA. All these facts make abundantly clear the functional significance of residual protein. ALLFREY *et al.* (1955b) see in it the locus of cytoplasm-chromosome interaction right within the chromosome. In the polytenic chromosomes, directly participating in synthetic processes across the nuclear membrane (GAY 1956), if such a dynamism of the residual protein obtains it would be difficult to dissociate from the function of genes. A likely co-participant would be RNA, which in these chromosomes shows at this stage great metabolical activity at a time when DNA does not (PELC and HOWARD 1956); the active RNA is mostly in the bands. In *Chironomus* the protein metabolism is higher in bands rich in RNA (FICQ and PAVAN 1957).

In its samples each chromosome shows a variability in the total protein sulphur which is not surprising in biological material. On the other hand, evidence has been presented (e.g. the tendency of different cells of the same larva to show a constantly low or high content for all chromosomes) which indicates probable developmental variations. These may be related to the position of the cell in the gland, i.e. apex versus fundus; salivary gland cells grow vegetatively during almost all the larval life and fundal cells are at all times larger in their components than apical cells (DEMEREC 1950). If size and function increase together in the salivary cells, which is not proved, then the above interpretation would be in keeping with the correlation between amount of residual protein and physiological activity already found (MIRSKY and RIS 1951).

In cell 57.1 the high protein-sulphur content in chromosome X and lower content in the others could conceivably have arisen through unequal polyteny (due to shifts of temperature?). Some such morphological evidence has been gathered in Diptera (MELLAND 1942) and is discussed by ALFERT (1954). A situation like this could have escaped notice in measurements for whole nuclei, which is a frequent practice in cytophotometry.

As to the activity resolved as ARGs, the difference between it and the unresolved activity in the rest of the chromosome is largely one of sulphur concentration and this is greater in the former. Sulphur concentrations mean that residual protein is more concentrated or that, in terms of sulphur, it is regionally differentiated. This residual protein

could be associated with either DNA (in bands) or RNA (in bands and interbands). For expediency, in what follows we preserve the term of "ARGs" for the residual protein agreeing with this description: they have been plotted at a subdivisional scale. The pattern of ARGs of bands and interbands is much less uniform than that of the overall activity. But certain bands and interbands show activity more repeatably than others. Evidence was presented to the effect that these ARGs, and their pattern, are largely real. Nonetheless, all randomizing factors will impinge more heavily on the resolved ARGs than on the total activity of the chromosomes. It cannot be excluded that differences in development may be operative in producing the pattern of ARGs.

The intensity of individual ARGs in an extensible object, such as chromosomes are, is not a reliable index for correlations. It is not obvious that reduction of the variance in stronger, longer exposed ARGs could have increased the repeatability between samples (without affecting the uniformity of the overall activity). Exposures of the chromosomes for 5, instead of 3, days still show a very discontinuous localization of ARGs. Stronger ARGs, by obscuring the chromosome, would seem impracticable unless for short, well defined regions. Specially interesting should be the comparison of our data on the distribution of residual protein with similar quantitative data on the turnover of this protein (by short labelling of late larvae) along the chromosomes. It would not be surprising if the pattern of ARGs corresponds with a pattern of turnover rates along the chromosomes. PELC and HOWARD (1956) found that the labelling of 'intact chromosomes' with methionine-^{35}S, for shorter times than ours, was uneven along the chromosomes. Due to the expected amount and metabolism of residual chromosomes much of this label should be in them. 'Intact chromosomes' labelled *ab initio* with methionine-^{35}S show as much discontinuity of labelling as the residual chromosomes (unpublished).

With few exceptions the ARGs are not reflected in the curves of cumulative activity in our scale of plotting. The independence is explained by labelled chromosome protein contributing activity which, if not disposed as to be resolved as ARGs, buffers any effect of the ARGs on the curves. All major chromosomes are reasonably comparable in the proportion of the number of ARGs to the total activity, the latter being proportional to chromosome size. Chromosome 2 R shows a lesser proportion (27% less): its activity is made more of unresolved activity than that of any other arm. All major chromosomes have the same percentage of the overall activity resolved as ARGs, except 2 R, with a lower percentage (20% less). These latter measurements show a reliable constancy for biological material. With chromosome 4 R, due

to its small size and activity, this was not so and without serious error it can be neglected. The mean intensity of ARGs being equal in all chromosomes, the activity as ARGs is proportional to the number of ARGs. All chromosomes, except 2 R and 4 R, have 22% of their subdivisions with ARGs (656 out of 2,969 subdivisions effectively scored) and 50% of their activity as ARGs; chromosome 2 R has 16% of the subdivisions with ARGs (126 out of 779) and 40% of the activity as ARGs (expected: 36.4%). The mean activity of subdivisions with ARGs is 4.5 photographic grains (Table 6) and, of subdivisions without ARGs, 1.2 grains.

The combination of what has been said for the overall activity and for the activity as ARGs results in two patterns of concentration of residual protein coexisting in these chromosomes: a pattern at a divisional scale of plotting which is not sharply differentiated and a pattern at a subdivisional scale which is considerably, but not entirely, variable. The divisional pattern is the same for all the chromosome map. Computed by its high peaks — the ARGs — the subdivisional pattern is in all chromosomes of an equal size relative to the whole, except for chromosome 2 R, where it is smaller. The existence of the subdivisional pattern is deduced from: 1. the reality of the ARGs on which it is based, 2. the correspondence of the ARGs with bands and interbands, 3. its constant size over all samples of a chromosome, and 4. its lesser size in one chromosome compared with the rest of the complement.

The possibility that the whole of the subdivisional pattern is due to unextracted histones, unevenly distributed along different samples, is very slight: the sulphur of sites with ARGs is half the total sulphur in the hydrolysed chromosomes. According to MIRSKY and RIS (1951) the salivary chromosomes, being in a cell with abundant and active cytoplasm, could be predicted as rich in residual protein. Even in the intact chromosomes twice as much histone as residual protein[1] would be necessary to account for 50% of the joint sulphur as histone sulphur. It is unlikely that there is that much histone in the intact polytenic chromosomes. Furthermore, though histones are mostly confined to bands (HORN and WARD 1957) ARGs of interbands are frequent.

The significance of the subdivisional pattern is less easy to assess. Considering that it measures a variable of concentration along length, and that the high concentrations at the peaks (ARGs) and the low concentrations at the troughs (non-resolved activity) cancel each

[1] The three sulphur-aminoacids have a similar percentage of sulphur. The average of 17 normal histones listed by BLOCK and WEISS (1956) is 0.92% sulphur-aminoacids, and of 4 other histones (STEDMAN *et al.* 1957, and pers. com.) from 0.4 to 1.6%. The average of 2 chromosomins (STEDMAN, pers. com.) is about 2% sulphur-aminoacids.

other at the divisional scale, one question is posed: does the pattern mean statistical variation or does it mean specific variation? The answer is in its size. The only reliable measurement that we have of this pattern is the ratio of activity in its peaks to the overall activity. This largely excludes any personal bias in the recognition of the ARGs: it is statistically uniform and high (50%) in all samples except 2 R. As to the size of the location of these peaks, which includes the bias in the scores, it is 22% of the subdivisions sampled[1] (for chromosome 2 R the corresponding percentages are 40 and 16). Only if the latter were a much higher percentage could the size of activity of the peaks be taken as random variation within the total activity. As it is, no statistical property inherent in the technique can account for such results in the absence of a real phenomenon. On the other hand, it is difficult to see how such results could be brought about by, for instance, changes in the ratio $^{35}S:^{32}S$ of the protein precursors. The pattern should be taken as a measure of specific variation. On the average, differences of four times or less the concentration of ^{35}S were detectable with the technique. One grain associated with a band will not be scored as an ARG; five associated grains can be scored as an intense ARG: the first corresponds to 66 ^{35}S-atoms at fixation, the second to 330.

In conclusion: at the divisional scale of plotting there is a rather uniform distribution of residual protein (a metabolically significant protein) along the entire chromosome complement, and most probably a constant fraction of that amount (except for chromosome 2 R) is considerably non-uniform as measured by its sulphur at the subdivisional scale. That the functional unit exhibiting this non-uniformity is contained within subdivisions has been substantiated in a few scores within subdivisions (p. 133). *A priori* one could expect residual protein, being a quasi-continuous component, to show a less localized function than a discontinuous component such as DNA. The conclusions on the non-uniform component are, we are aware, conditioned by the limitations of the technique.

The emerging picture of the chromosome at the submicroscopic level is devoid of many of the entities, i.e. bands and interbands, with which one is familiar with the light microscope. The functional unit in our work will comprise assemblies of the submicroscopic units proposed by Lowman (1956): rods of residual protein with terminally attached nucleoprotein granules, the whole measuring 800 Å on the average, forming continuous strands (chromonemata) along the chromosome. The resolving limit in our technique is half a subdivision (average

[1] Estimate for all chromosomes but 2 R and 4 R (see p. 149). This is an outside estimate as ARGs of part of subdivisions were scored by the full subdivisions.

medium-stretched length: 1 μ) corresponding to 12,800 submicroscopic units $\left(\frac{10{,}000\ \text{Å} \times 1{,}024\ \text{chromonemata}}{800\ \text{Å}}\right)$ in extended chromonemata.

Three simple explanations of the subdivisional ARGs can be given. (a) Net increase of residual protein in those subdivisions, which is subsequently released from the residual chromosome (as gene products ?). The possibility of localized enrichment in sulphur of the residual protein deserves mention. (b) Migration along the chromosome, e.g. towards the nucleolus via the chromocenter, of discrete chunks of residual protein (this was suggested to us by Professor C. H. WADDINGTON). (c) Localized, reversible folding of strands (in waves ?), which were originally folded and later partially unfolded (ALFERT 1954) along the chromosome: increased folding would mean more strand material per site. Sites of high repeatability of ARGs would be, for (a), where new protein is more frequent; for (b), as for (a) or where migrating protein remains stationary; for (c), where folding is more frequent, or where waves converge or remain stationary. Any of these three explanations has to account for an almost fourfold increase of protein (see p. 149) at the sites of the ARGs. The independence of the subdivisional pattern on the actual length of the chromosome makes it unlikely that this pattern could be due to an artifact of fixation at the submicroscopical level.

All three explanations hold in terms of the residual chromosome as a whole, rather than in terms of parts, because of the constant size-variable location of the ARGs. In particular, the explanations by migration of protein (b) or by waves of folding (c) help visualizing a control of parts of the chromosome by the chromosome as a whole, without that necessarily making any one of them the true explanation. If explanation (b) proves correct it would go against all the non-histone protein of the chromosomes, after our procedure, being formed of strands. After reaching our conclusions we became aware that RIS (1957, p. 55) had already proposed explanation (a) for the microscopically observable variation of size in the loops of lampbrush chromosomes and in the puffs of polytenic chromosomes: unit fibrillar particles would be interstitially added in, or removed from, these regions of the chromosomes. If applicable to our observations this interpretation would be extended to the submicroscopical level. The possibility of localized folding has been recognized by ALFERT (1954, p. 154) (see also SCHMITT 1956). Possibilities (a) and (c) could be implicit in suggestions made by SCHULTZ *et al.* (1955) on reversible, localized swelling of the chromosomes. For a highly metabolical protein, such as residual protein, localized increases of protein could result in a parallel pattern of rates of incorporation verifiable after short labelling of late larvae. It is ob-

vious that the explanations advanced result in different meanings for the phenomenon described, and further work is contemplated to elucidate between these explanations.

The less variable subdivisional pattern of chromosome 2 R remains to be explained. This chromosome differs from the others in three respects; if ultimately these should have to be seen as only two, or perhaps one, is irrelevant at the moment. First, the density of bands and of interbands (not the total number) is greater (BRIDGES 1942). The density is 19% greater than that of all other chromosomes combined (but only 6% greater than X's); this is also the order of the decrease in size of the subdivisional pattern. Secondly, and in accord with the first, more genes have been found in this chromosome than in any of the other autosomes (BRIDGES and BREHME 1944); the number in chromosome X is greater but this may reflect the greater ease of detection of sex-linked mutations. Thirdly, it contains less intercalary heterochromatin (HANNAH 1951), when judged by ectopic pairing, breakability and repeats, but not when judged by the 'minute' genes (whose relation to heterochromatin is, however, unsettled). Thus, in our scores the less variable pattern of chromosome 2 R could be related to its greater chemical differentiation or to its relative poverty in heterochromatin. If the latter were to obtain, though the variable pattern may originate in the intercalary heterochromatin, it need not mean that in the other chromosomes it is seated in the heterochromatin itself. Heterochromatin is the one chromosomal component endowed by tradition with long-range effects. Nor has it been possible in any way to relate unequivocally the subdivisional pattern of the chromosomes with the distribution of heterochromatin, as figured by HANNAH.

Intercalary heterochromatin is well defined in chromosome X by its protrusions, and much less so in other chromosomes (HANNAH 1951). There was no clear-cut correlation between overall activity or ARGs and heterochromatin in chromosome X. But, in general, heterochromatin being contained within single subdivisions or so, its behaviour is bound to be masked by the scale of plotting. For these reasons the activity of heterochromatin is best assessed in chromocenters, heterochromatic in these cells. If it is considered that the chromocenter is where all chromosomes interfuse (two of them are metacentric) (see EMMENS 1937, his fig. 3a), the quantity of protein should be greater than for the same area in single chromosomes, and therefore its specific activity of 0.66 grains/μ^2 becomes an overestimation when used for comparisons. Greater thickness of the chromocenters was, however, not directly observed; they certainly were not overstretched. This would indicate a low protein sulphur content compared with the chromosomes of specific activity 0.56 grains/μ^2. This low sulphur content in the chromocenter is in accordance with the diminished residual protein

found by staining and enzymatic procedures (SCHULTZ 1947) or by the reduced number of submicroscopic strands (LOWMAN 1956). On the other hand, the UV-absorption of 'intact' chromocenters points to a sizeable amount of total protein (CASPERSSON 1950).

A structural function could be envisaged for the sulphur in the residual protein (a structural protein itself) due to its uniform distribution at the divisional scale. But this necessitates its presence as cyst(e)ine, which is absent in some chromosomins studied (STEDMAN, pers. com.) but not in others (STEDMAN 1943; WANG *et al.* 1953). The possibility is given by the well known existence of -SS- bridges inside and between protein molecules. More recently a special capacity of SH groups for hydrogen-bonding has been proposed (FRAENKEL-CONRAT 1956). Some experimental facts are available which, if not unequivocally interpretable (due to pH's, etc.), are in line with our proposition. Disruption of polytenic chromosomes follows after treatments with thioglycollate (MAZIA 1955), which reduces disulphide bonds, or with performic acid (DANIELLI 1947), which oxidizes them. MAZIA (1954) claims that macromolecular nucleoprotein fragments of the chromosome are bridged together by divalent cations and other ionic forces. AMBROSE (1956) ascribes to H-bonds the longitudinal cohesion of chromosomes, and to the electrostatic set-up the lateral cohesion. It may be significant that histones, with the least sulphur content of all proteins in somatic chromosomes, are not structural proteins. It is realized that disulphide bridges can play only a restricted part as these chromosomes are highly elastic, and a lattice built entirely on such bridges would be rigid (FREY-WYSSLING 1953). In our material, the decay of ^{35}S into ^{35}Cl, involving a change of valence and probably a rearrangement of molecules, should not in the envisaged scheme, and did not, affect the integrity of chromosomes sampled for chromosome mutations at day 21: the total sulphur is several orders of magnitude higher than the decayed sulphur[1].

Comparison between polytenic chromosomes and a metaphase chromosome

In one metaphase chromosome from root tips of *Vicia faba* the distribution of ^{35}S in residual protein[2] was studied (PELC and HOWARD 1952). This chromosome has a submedian heterochromatic centromere

[1] Ratio of label in tracer at day 21:91: 8.9×10^4. Decay of tracer during 20 days in salivary gland: ca. 15%. Ratio of total to decayed sulphur: $\frac{8.9 \times 10^4}{0.15}$ = order of 10^5. The unknown dilution of tracer by sulphur in the medium will increase the above ratio.

[2] In a similar material (onion root) this residual protein, as measured in whole nuclei, is given as 50% of the total protein (RIS 1947). Because of loss of proteins and other reasons connected with the technique, this value is probably overestimated.

and its longer arm carries a nucleolar organizer. The chromosome is 22 μ long and eight samples were plotted in segments of 3.5 μ: grain counts showed a repeatable pattern. In the shorter arm the maximum number of grains was at or near the free end and the number fell noticeably towards the centromere; in the other arm, from the centromere till past the nucleolar organizer that number remained constant and rose sharply at the free end to about the same number as in the end of the other arm (*ibid.*, Fig. 2). The incorporation of tracer represented synthesis and not exchange. The samples of the *Vicia* chromosomes were labelled during one duplicative cycle. From the similar grain counts per segment of chromosome between samples, it is quite obvious that independently of the type of duplication of this protein, this is the pattern of its relative sulphur content along the chromosome. To decide if the new protein is entirely in one or in both of the two chromatids forming the chromosome, chromatids have to be analysed when segregating as chromosomes at the following mitosis.

The pattern of sulphur in the *Vicia* chromosomes is the equivalent of the overall pattern of the polytenic chromosomes and should be compared with it. The bimodal pattern was explained in, for simplicity, two opposed ways: either there is more residual protein at the end of both arms, or, this protein is there richer in sulphur. What is relevant is that when comparing the pattern of the two arms of the metaphase chromosome with that of the two arms of the metacentric polytenic chromosomes (chromosomes 2 and 3) the first type are unimodal and the second type are uniform (all plotted in segments $^1/_7$ to $^1/_9$th the length of the chromosome). These differences can hardly be due to chance alone and must indicate a difference in organization, on which, until more comparisons of the kind are at hand, speculation is unfruitful.

The protein sulphur of other cell components after hydrolysis

For the comparisons in this section certain considerations are necessary. The recovery of grain counts and the surface estimations are better for nucleoli than for chromocenters and chromosomes. The specific activity of chromosomes was calculated using averages of maximum activity, of lax length, and of width. For the other two components direct measurements were made. Equal thickness after squashing had to be assumed for all three components: this yielded a reasonably consistent specific activity for the three. It is obvious then that the comparisons can point no more than to the order of differences.

Nucleoli would also have their histones hydrolysed. In fact the chemical determinations cited before (Stedman and Stedman 1950), showing a disappearance of histones after hydrolysis, refer to whole

interphase nuclei with their nucleoli included. The specific activity of nucleoli (0.51 grains/μ^2) is of the same order as that of chromosomes (0.56 grains/μ^2). In other words, the amount of sulphur in their non-histone protein is alike. How much this residual protein of nucleoli resembles that of chromosomes as a whole ist not known. Under the electron microscope, nucleoli lack the fibrillar structure of the chromosomes (LOWMAN 1956); on this account a difference in the residual proteins would be expected. If the concentration of total proteins of intact nucleoli is higher than that of chromosomes (a situation which, in general, is most likely) and if the sulphur content of both proteins is similar, one could think that more protein has been extracted during the procedure from nucleoli than from chromosomes. Qualitative observations (HORN and WARD 1957) do however show that there is more histone in the chromosomes than in the nucleolus, so that on this basis one could provisionally conclude that the latter contain less sulphur in their non-histone proteins. But it must be remembered that the different staining intensities found by HORN and WARD could be due to differences in the amount of groups available for the reaction rather than to actual differences in the amount of histones.

When the specific activities of nucleoli (0.51 grains/μ^2) and chromocenters (0.66 grains/μ^2) are compared — a comparison which is relevant because the latter includes the nucleolar organizer — the relative protein sulphur after hydrolysis is as great (or greater, because of a possible overestimation for chromocenters) in the nucleolus as in the chromocenter. Histones present both in the nucleolus and in the chromocenter (HORN and WARD 1957) would have been hydrolysed. The matrix of the nucleolus resembles that of the chromocenter in its poor fibrillar structuration (LOWMAN 1956). Comparisons between cell components such as in this and in the former paragraph were beyond our initial scope and remain inconclusive. For these comparisons at least a gross fractionation of proteins is necessary, i. e. combined with cytophotometry (POLLISTER and RIS 1947). This was already made plain in the revision of the literature.

Considering chromosomes and nucleoli, the apparent similar order of sulphur content in their non-histone proteins emphasizes the sharp differences found always in favour of nucleoli for the rates of incorporation into proteins (reviewed in SIRLIN and WADDINGTON 1956). In this work we have dealt with female nucleoli, and these have a much higher relative protein content than those from males (SCHULTZ *et al.* 1940); the discrimination of sex is rarely made in the literature on the subject. If the fact that in *Drosophila robusta* the nucleolus commences to regress at mid-third instar before the nucleus has attained its maximum size (LESHER 1951) is of general occurrence, it is bound to have implications.

The granules described in the cytoplasm are connected with the secretion of the puparial glue (Gay 1956). They contain mucoprotein which could have been partly lost from the preparations. Thus they could have incorporated tracer either as aminoacid into their protein moiety, or, as sulphate derived from the aminoacid (Melchior and Tarver 1947) into the prosthetic moiety. The latter possibility is unlikely according to other work (Pelc and Howard 1956). According to the age of the larvae used here it seems probable that the absence of granula in some of the larvae represents their dissolution and discharge into the lumen.

Summary

Salivary gland chromosomes of *Drosophila melanogaster* have been labelled by feeding with methionine-^{35}S throughout larval life. This provides a measure of relative concentrations of protein sulphur. After Feulgen hydrolysis the chromosomes were studied by means of high resolution autoradiography. It is believed that the main protein studied is residual (non-histone) protein of the chromosome, and provisional interpretations have been made on this basis. This protein is highly metabolical active.

The activity was scored, by subdivisions, as unresolved activity or as autoradiographs resolved for bands and/or interbands. The difference between them is one of concentration of protein sulphur, which is about four times higher in the autoradiographs.

The following points have emerged:

1. When plotted by the divisions of Bridges' chart the concentration of protein sulphur is uniform along the whole chromosome complement. Some samples of a chromosome, however, have considerably less protein than other samples of that chromosome.

2. When plotted by subdivisions, the repeatability of the occurrence of autoradiographs in the same subdivisions for different samples of a chromosome is variable and not high.

3. In all chromosomes, except 2 R, the activity as autoradiographs resolved for bands and/or interbands is 50% of the total activity. In chromosome 2 R it is 40%.

4. From approximate comparisons it results that the mean intensity of autoradiographs is the same over all the chromosome complement. The lesser activity of chromosome 2 R as autoradiographs is due to the lesser number of subdivisions with autoradiographs relative to the total activity of the chromosome.

In conclusion, the protein sulphur of the chromosome includes a fraction represented by local increases in concentration. The location but not the relative size of this fraction is variable indicating that this fraction is under control of the chromosome as a whole. Three explanations are offered, between which it is at present impossible to decide.

These locally increased concentrations can be due to: (a) net increase of protein (or of protein sulphur but not of protein itself), or (b) increased submicroscopic folding of the chromosome. The constant relative size is suggestive of: (a) rapid release of new protein from the chromosome, or (b) displacement of protein along the chromosome, or (c) waves of folding.

5. Heterochromatin in the chromocenter probably shows lesser relative protein sulphur than euchromatin.

6. Nucleoli show the same relative protein sulphur in their non-histone proteins as chromosomes.

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CHROMOSOMALER STRUKTURPOLYMORPHISMUS BEI EINER ZAPRIONUS-ART

Von

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Der Verfasser beschrieb (MAINX 1958) eine neue Drosophilide aus Java, die auf Grund verschiedener morphologischer Merkmale zur Gattung *Zaprionus* gestellt wurde und den Namen *Z. bogoriensis* erhielt. Die Gattung ist allerdings bisher nur aus Afrika bekannt, wo sie einen großen Artenreichtum zeigt. Auch in der Zahl der Eifilamente stimmt die neue Art nicht mit den bisher so weit bekannten *Zaprionus*-Arten überein. Es wird vielleicht später die Begründung einer neuen Gattung für diese auffallende, große Drosophilide nötig sein. *Zaprionus bogoriensis* scheint auf Java häufig zu sein und wurde dort wiederholt auf Früchteködern in weiblichen und männlichen Exemplaren gefangen. Sie ist auf dem üblichen *Drosophila*-Nährboden sehr leicht zu züchten.

Die Mitose wurde an den Neuroblasten und in den Imaginalscheiben der erwachsenen Larven untersucht. Es zeigen sich 5 Paare stabförmiger, akrozentrischer Chromosomen und 1 Paar kleiner, kugelförmiger. Die Homologen liegen stets streng parallel und eng benachbart angeordnet. Das eine stabförmige Chromosomenpaar ist deutlich länger als die anderen 4 unter sich gleich langen Paare. Es ist wahrscheinlich das Geschlechtschromosomenpaar. In männlichen Larven konnte kein Unterschied zwischen dem X- und dem Y-Chromosom festgestellt werden, bis auf einen geringen Unterschied in der Färbbarkeit in Essig-Orcein. Die Interphasekerne der Imaginalscheiben zeigen einen meist zweilappigen, stärker färbbaren Körper, vielleicht das heterochromatische Y-Chromosom. Die Riesenchromosomen sind in den Speicheldrüsen der erwachsenen Larven sehr gut ausgebildet, aber stark tordiert, so daß sie bei der Präparation leicht brechen oder kurze Schlingen bilden, die mit Inversionen verwechselt werden könnten. Es ist ein deutlich ausgeprägtes Chromozentrum von grobkörniger Beschaffenheit vorhanden, von dem die fünf langen Elemente ihren Ausgang nehmen. Bei der Präparation lösen sie sich leicht aus dem Chromozentrum, in das sie von ihrem proximalen Ende aus mit dünnen Fäden einstrahlen. Der große Nukleolus zeigt ein färbbares Zentralkörperchen, das oft mit einem dünnen Faden mit dem Chromozentrum zusammenhängt. Das X-Chromosom ist bei männlichen Larven schwächer gefärbt aber nicht

dünner als beim Weibchen und bleibt mit seinem proximalen Ende stets mit der Hauptmasse des Chromozentrums verbunden. Ein Autosom trägt einen kleinen Nukleolusbildner (Balbianiring). Kleinere und größere Paarungslücken zwischen den Homologen sind ziemlich häufig. Es kommt auch vor, daß die Homologen nur an den beiden Enden oder nur am distalen Ende im Bereich einiger Querscheiben zusammenhängen.

Es wurden 27 Präparate eines Stammes auf Inversionen geprüft, der in Jogjakarta (Java) isoliert worden war. In 11 Präparaten wurde ein Komplex aus zwei übergreifenden Inversionen festgestellt, der in der Nähe des proximalen Endes eines Autosoms liegt und fast die Hälfte der Länge dieses Chromosoms einnimmt. Die beiden Inversionen übergreifen einander nur über einen kurzen Abschnitt. Das Bild des Komplexes ist oft durch mangelhafte Paarung der Homologen gestört. Außerdem wurde in 2 Präparaten eine große, einfache, mittelständige Inversion in einem anderen Autosom gesehen, die fast die Hälfte der Chromosomenlänge einnimmt. In einem Präparat kommen die beiden Inversionstypen gemeinsam vor. 15 Präparate zeigten keine Inversionen. Von einem anderen Stamm, der in der Umgebung von Bogor (Java) geködert worden war, konnten 4 Präparate geprüft werden. In 2 Präparaten zeigte sich der gleiche Komplex aus zwei übergreifenden Inversionen. Die beiden Strukturtypen, die zusammen das Bild der beiden übergreifenden Inversionen geben, scheinen also, zumindest auf Java, weitverbreitet zu sein. Daß zwei Stämme, bei deren Begründung nur von einem oder sehr wenigen Weibchen ausgegangen wurde, chromosomal so polymorph sind, zeigt, daß auch bei dieser Art ein stärkerer chromosomaler Polymorphismus der natürlichen Populationen angenommen werden kann, wie er bisher für viele *Drosophila*-Arten und andere Dipteren nachgewiesen wurde (letzte Zusammenstellung Mainx 1956).

Zum Vergleich wurden die zytologischen Verhältnisse von 3 afrikanischen *Zaprionus*-Arten untersucht, die in den Stämmen der Kulturensammlung in Pavia zur Verfügung standen. *Zaprionus vittiger* wurde 1952 in Abidian, Goldküste, isoliert, *Z. tuberculatus* 1955 in Zoutpansberg, Süd-Afrika, während der Stamm von *Z. ghesquièrei* unbekannter Herkunft ist. Alle 3 Arten zeigen in der Mitose das gleichförmige Bild von 5 stabförmigen, akrozentrischen, ungefähr gleich langen Chromosomenpaaren und einem kleinen, kugelförmigen Paar. Ein Unterschied zwischen X- und Y-Chromosom ist nicht feststellbar. Es ist dies die „Basisanordnung" des Chromosomensatzes, die auch bei manchen *Drosophila*-Arten vertreten ist und die bei der Gattung *Zaprionus* verbreitet zu sein scheint. Auch dieser Umstand spricht für die Zuteilung der neuen Art *bogoriensis* zur Gattung *Zaprionus*. Je 20 Speichel-

drüsenpräparate der 3 Arten wurden auf Inversionen geprüft. Während bei *Z. vittiger* und *Z. tuberculatus* keine Inversionen gefunden werden konnten, wurde in 3 von 20 Präparaten von *Z. ghesquièrei* eine kleine Inversion in einem langen Autosom gesehen. Dieser Befund an Laboratoriumstämmen schließt es natürlich nicht aus, daß in den natürlichen Populationen dieser 3 afrikanischen *Zaprionus*-Arten chromosomaler Polymorphismus vorkommt, doch dürfte er nicht sehr ausgeprägt sein.

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THE CHROMOSOME COMPLEMENT OF THE RHESUS MONKEY (MACACA MULATTA) DETERMINED IN KIDNEY CELLS CULTIVATED IN VITRO*

By

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With 3 Figures in the Text

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Introduction

As part of a cytological study on tissue culture strains designated as "altered monkey kidney" (AMK) by Parker *et al.* (1957), it was desirable to study the chromosome complement of normal Rhesus monkey.

The chromosome number of this monkey has previously been reported as 42 by Darlington and Haque (1955) and Chu and Giles (1956). The former workers have also identified the Y chromosome[1]. The present paper describes studies on the chromosomes of monkey kidney cells cultivated *in vitro*, and extends the analysis of chromosome morphology to the point where most chromosomes of the complement can be individually identified.

Preparation of cell suspension and slides

Details of the preparation of cell suspensions and their *in vitro* propagation have been described elsewhere (Siminovitch, Rothfels and Parker). In general, cultures were prepared by trypsinization of kidney cortex from wild adult Rhesus monkeys of known sex, obtained from India. The trypsinized cells were dispersed into a medium consisting essentially of lactalbumin hydrolysate, yeast extract, 6% ox serum and salts (Cooper 1955). Twenty ml. aliquots of properly diluted suspension were placed into 10 cm diameter Petri dishes each containing two slides. The Petri dishes were kept at 37° C and flushed continuously with air containing 5% CO_2. Viable cells settled within 12 hours and began to proliferate on the glass. The medium was changed after 36 to 48 hours and cells were profitably examined three to six days after explantation.

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[1] While this paper was in press "A study of primate chromosome complements" by E. H. Y. Chu and Norman H. Giles appeared [Amer. Natur. **91**, 273—282 (1957)] in which a photograph and some description of the chromosome complement of *M. macaca* is given.

Suitable slides, with a rather sparse growth of actively multiplying cells were pretreated for 30 minutes with a one quarter strength Tyrode solution (HSU 1952). Fixation followed in acetic alcohol (1:3) for 10 minutes. Following fixation, slides were allowed to dry completely and this resulted in a flattening of the cells and separation of the chromosomes without undue scattering. Large numbers of intact metaphases in which the chromosomes were well spread were thus obtained without manual squashing. The preparations were stained in 2% natural orcein (G. T. GURR, London) in 50% acetic acid and examined as temporary mounts or in euparal following a dry ice mounting schedule (CONGER and FAIRCHILD 1953). These cytological techniques are described more fully elsewhere (ROTHFELS and SIMINOVITCH, 1958).

Chromosome number

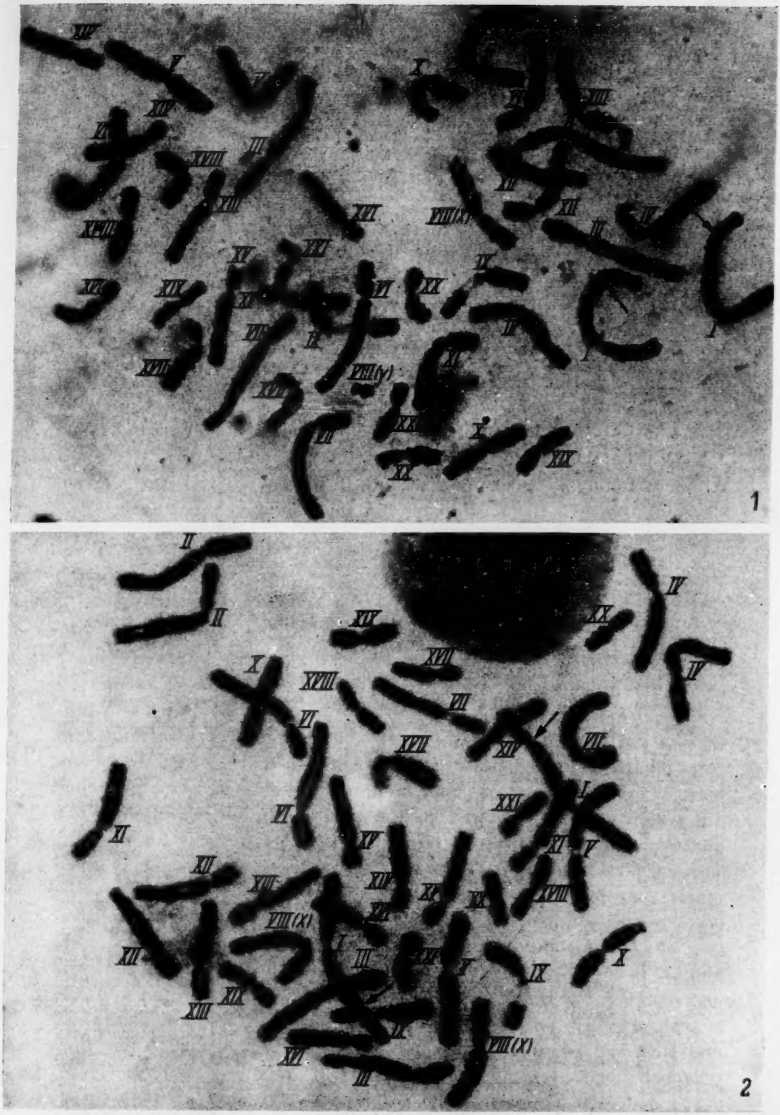
In suitable preparations the bulk of the divisions were clearly normal and diploid. The rare polyploid nuclei and the more common endoreduplicated ones (LEVAN and HAUSCHKA 1953) will not be considered in this paper since they will be described elsewhere (SIMINOVITCH, ROTHFELS and PARKER). The vast majority of the non-polyploid cells had exactly 42 chromosomes. In one of the better slides, one hundred consecutive (i. e. non-selected) metaphases were counted for chromosome number. Ninety seven were found to be 42. One, with a count of 43 proved to have a chromosome broken through the centromere, possibly during preparation of the slide. The remaining two nuclei had 41 chromosomes. Occasional counts of 41 were also made in apparently intact cells in other slides. These appear to be genuine and presumably arise through non-disjunction. In good slides no counts lower than 41 were found.

Chromosome measurements

In order to obtain measurements for the quantitative description of chromosome morphology, metaphases are required in which the chromosomes are well spread, with clearly defined centromeres, and free of overcontraction and obvious differential stretching. Nuclei from a male (Fig. 1) and from a female (Fig. 2) approximating this condition and actually used in analysis are shown as examples.

Once suitable nuclei were selected, tracings were obtained, either as camera lucida drawings of individually centered chromosomes using a 20 × ocular (LEVAN and HAUSCHKA 1953); or, from prints or projections of 35 mm negatives taken on Dupont Microcopy. In our experience tracing of negatives projected in a micro film reader was the least laborious and most accurate of the methods tried.

In the tracings of chromosomes, the two chromatids were treated separately. The ends of the chromosomes were usually well defined but the centromeres, which formed non-staining gaps of considerable extent, required a standardized treatment.



Figs. 1 and 2. Photomicrographs of nuclei, in metaphase, from Rhesus monkey kidney cells cultivated *in vitro* (× 1520). Permanent mounts. 1 from male, 2 from female

The centromere gap was partitioned equally, and line tracings were made as accurately as possible from the insertion point of the chromatids down the center of each chromatid to the ends. Tracings of long and short arms were measured separately with a map measurer (Dietzgen, Switzerland) calibrated in eighths of an inch. Measurements were estimated to the nearest tenth division and for each chromosome the mean of the two short, and the two long, chromatid arms was recorded in "units". These measurements formed the primary data.

In monkeys, the males are heterogametic (XY), and their nuclei may always be recognized by the extremely small Y chromosome which is also the only acrocentric of the complement (see Figs. 1 and 2). The X chromosome, identified by elimination, is a medium sized metacentric, eighth in decreasing order of size and typically with an arm ratio of about 1.4[1]. The chromosomes were numbered by Roman numerals in decreasing order of size, with minor modifications so as to group together chromosomes with similar arm ratios. It may be noted that there is a tendency for homologous chromosomes to lie together. A quantitative study of this somatic pairing was not undertaken.

Identification of homologous chromosomes

Table 1 shows the analysis of a single nucleus (♀4) to illustrate in a typical specimen the further treatment of the data. In order to give all nuclei equal weight, measurements of chromosome arms in "units" were recalculated as a per cent of the total diploid complement length (% diploid TCL) for each nucleus. For each chromosome the arm ratio was calculated by dividing the length of the short arm into that of the long arm (units). Finally chromosomes were arranged in pairs on the combined basis of relative length and arm ratio. As can be seen from Table 1 this could be done with ease for some pairs but difficulties were encountered in the characterization of others. For instance, the chromosomes numbered I (I_1 and I_2) clearly form a pair. Their arm ratios are identical and even the unusually short I_2 is much longer than any other chromosome of the complement. Similarly the chromosomes numbered IV form a convincing pair. But the identification of the four intervening chromosomes which constitute pairs II and III is contentious because of conflicting indications from arm ratio and total length. Again, the proposed association of pairs XI and XII is arbitrary. All remaining pairings in this nucleus are straightforward, and with the possible exception of pairs XIX and XX, even unequivocal.

It should be pointed out that chromosomes which are difficult to pair in one nucleus may be classified unambiguously in another and

[1] For convenience, the arm ratios are written as indices, e.g. 3:1 as 3.

Table 1. *Analysis of sample nucleus* (♀ 4). *Identification of homologous chromosomes by length and arm ratio*

Chromosome number		Short arm (s) Units	% TCL	Long arm (l) Units	% TCL	Total % TCL	Arm ratio l/s
I	1	6.5	1.66	12.0	3.05	4.71	1.84
	2	5.4	1.38	9.9	2.52	3.90	1.83
II	1	4.5	1.15	9.0	2.29	3.44	2.00
	2	4.1	1.04	9.4	2.39	3.43	2.29
III	1	3.6	0.92	9.7	2.47	3.39	2.69
	2	4.3	1.09	9.0	2.29	3.38	2.09
IV	1	4.1	1.04	8.5	2.16	3.20	2.07
	2	4.0	1.02	8.2	2.09	3.11	2.05
V	1	5.0	1.27	6.9	1.76	3.03	1.38
	2	5.2	1.32	6.5	1.66	2.98	1.25
VI	1	3.1	0.79	10.0	2.54	3.33	3.22
	2	3.1	0.79	9.8	2.50	3.29	3.16
VII	1	2.8	0.71	8.2	2.09	2.80	2.93
	2	2.9	0.74	8.0	2.04	2.78	2.76
VIII (X*)	1	4.5	1.15	5.3	1.35	2.50	1.18
	2	3.7	0.94	5.3	1.35	2.29	1.43
IX (N**)	1	5.0	1.27	4.5	1.15	2.42	0.90
	2	4.0	1.02	5.0	1.27	2.29	1.25
X	1	4.0	1.02	4.1	1.04	2.06	1.02
	2	3.6	0.92	4.0	1.02	1.94	1.11
XI	1	2.3	0.59	7.0	1.78	2.37	3.04
	2	2.4	0.61	6.8	1.73	2.34	2.83
XII	1	2.3	0.59	6.8	1.73	2.32	2.95
	2	2.6	0.66	6.4	1.63	2.29	2.46
XIII	1	2.3	0.59	6.1	1.55	2.14	2.65
	2	2.3	0.59	5.8	1.48	2.07	2.52
XIV	1	2.1	0.53	6.2	1.58	2.11	2.95
	2	1.8	0.46	6.0	1.53	1.99	3.33
XV	1	2.0	0.51	7.0	1.78	2.29	3.50
	2	1.9	0.48	7.0	1.78	2.26	3.68
XVI	1	1.5	0.38	5.8	1.48	1.86	3.87
	2	1.6	0.41	5.5	1.40	1.81	3.44
XVII	1	1.9	0.48	4.1	1.04	1.52	2.15
	2	2.0	0.51	4.0	1.02	1.53	2.00
XVIII	1	3.3	0.84	3.5	0.89	1.73	1.06
	2	3.3	0.84	3.4	0.87	1.71	1.03
XIX	1	2.3	0.59	2.9	0.74	1.33	1.26
	2	2.3	0.59	2.9	0.74	1.33	1.26
XX	1	2.2	0.56	2.8	0.71	1.27	1.27
	2	2.0	0.51	2.7	0.69	1.20	1.35
XXI	1	2.1	0.53	2.4	0.61	1.14	1.14
	2	2.0	0.51	2.4	0.61	1.12	1.20

* X: X chromosome.

** N: Chromosome bearing nucleolus in long arm.

the information derived from these more critical nuclei may help to resolve correctly the more problematic ones. It is probable that with greater stringency in the selection of nuclei complete and unequivocal pairing might always be attained.

Two minor modifications of this procedure were required to meet special situations. First, in order to make male nuclei directly comparable to female nuclei in % TCL, the X chromosome was included twice and the Y chromosome disregarded in the computation of total complement length in males. The length of the Y chromosome was then expressed in % TCL by applying the appropriate factor for changing measured length into % TCL units. Second, it appears that at least the X chromosomes and chromosomes XXI are dimorphic in the population analyzed, the alternative chromosomes agreeing in % TCL, but differing clearly in arm ratios. These discontinuities in arm ratio necessitated separate tabulation and treatment of the two X chromosomes (X and X′) and the two chromosomes XXI (XXI and XXI′).

Distribution of % TCL and arm ratio of homologous chromosomes in different nuclei

Five male and five female nuclei were selected, measured and analyzed for % diploid TCL and for arm ratio in the manner just described. The most salient data were mean % TCL and mean arm ratio for each chromosome.

Table 2 was drawn up to illustrate the actual distribution of % TCL and arm ratios of three chromosomes (I, II, III) for all ten nuclei. These chromosomes were chosen because I is clearly and individually distinct while II and III overlap broadly in the observed ranges of lengths and arm ratios. Thus Table 2 covers the extremes of the situations encountered in analysis.

The table shows surprisingly that for any chromosome pair the values of % TCL of the two homologues *within a particular nucleus* tended to be more alike than the values of any two such chromosomes chosen randomly from the measured population. Thus measurements of homologues from the same nucleus cannot be considered as entirely independent estimates. A probable explanation of this observation is that the degree of mitotic contraction differs between long and short chromosomes or chromosomes arms, so that % TCL of a given chromosome is to some extent a function of total complement length.

The table further shows that although there is some overlap in the observed range of % TCL and arm ratio for chromosomes I and II, chromosome I can clearly be distinguished from II and III in any one nucleus. In fact even chromosomes II and III, whose lengths and arm ratios overlap very widely, may be distinguished from each other with

Table 2. *Percent TCL and arm ratio in chromosomes I, II, III of 10 nuclei*

Nucleus	% TCL (diploid)			Arm ratio		
	I	II	III	I	II	III
1 ♀ 1	4.68	3.36	3.12	1.60	1.80	1.89
1 ♀ 2	4.32	3.24	3.00	1.57	2.00	2.57
2 ♀ 1	4.20	3.47	3.03	1.83	2.06	2.37
2 ♀ 2	4.09	3.41	2.98	1.54	2.13	2.07
3 ♀ 1	3.72	3.42	3.31	1.61	2.35	1.98
3 ♀ 2	3.60	3.41	2.97	1.77	2.26	2.09
4 ♀ 1	4.71	3.44	3.39	1.84	2.00	2.69
4 ♀ 2	3.90	3.43	3.38	1.83	2.29	2.09
5 ♀ 1	4.38	3.47	3.11	1.70	1.89	2.20
5 ♀ 2	4.21	3.33	3.04	1.60	1.94	2.24
1 ♂ 1	4.59	3.55	3.31	1.87	2.09	2.09
1 ♂ 2	4.19	3.37	3.21	1.76	2.15	2.11
2 ♂ 1	4.07	3.57	3.36	1.57	2.05	2.18
2 ♂ 2	3.81	3.47	3.18	1.70	1.78	2.21
3 ♂ 1	3.71	3.18	3.07	1.67	1.93	1.87
3 ♂ 2	3.71	3.16	3.01	1.67	1.96	1.98
4 ♂ 1	4.24	3.28	3.22	1.59	2.09	2.21
4 ♂ 2	4.10	3.25	3.10	1.36	1.80	2.20
5 ♂ 1	4.17	3.21	3.06	1.80	2.19	1.73
5 ♂ 2	3.95	3.17	2.95	1.62	1.88	1.98
Mean of 20	4.12	3.36	3.14	1.68	2.03	2.14

confidence in four of the 10 nuclei listed. Thus, with chromosomes like these, which would not be identifiable by their individual % TCL and arm ratio, a complete analysis of the nucleus may enable a definitive identification to be made.

Mean % TCL and arm ratio of all chromosomes

In order to construct an idiogram, the mean % TCL and mean arm ratio for all chromosomes was calculated. The results are shown in the last two columns of Table 3. The lengths are expressed in % haploid TCL rather than diploid since this in effect treats each chromosome pair as a unit and thereby acknowledges the unexpectedly close association in length of homologues within one nucleus. Graphical representation is also simplified in this way.

The separate values for males and females are also shown in the table. The comparison of male and female samples shows two trends. The mean arm ratio for chromosomes is generally higher in female nuclei than in male. The discrepancy is greatest in chromosomes VI, VII and XII—XVI with arm ratios larger than 2.5, and least in chromosomes I, V and XVIII—XXI with low arm ratios. A regularity is also

Table 3. *Mean % TCL (haploid) and arm ratio in 5 male and 5 female nuclei*

Chromosome number	Mean % TCL (haploid)		Mean arm ratio		Mean 5 ♂ + 5 ♀	
	5 ♂	5 ♀	5 ♂	5 ♀	% TCL haploid	Arm ratio
I	8.11	8.36	1.66	1.69	8.24	1.68
II	6.64	6.80	1.99	2.07	6.72	2.03
III	6.29	6.27	2.06	2.22	6.28	2.14
IV	5.78	5.90	1.80	1.92	5.84	1.86
V	6.08	6.40	1.23	1.33	6.24	1.28
VI	6.18	6.11	2.72	2.88	6.15	2.80
VII	5.86	5.65	2.82	2.98	5.75	2.90
VIII (X)	4.79 (5*)	5.24 (8*)	1.45 (5*)	1.38 (8*)	5.07 (13*)	1.41 (13*)
(X′)		5.17 (2*)		1.99 (2*)	5.17 (2*)	1.99 (2*)
(Y)	0.82 (5*)		6.50 (5*)		0.82 (5*)	6.50 (5*)
IX	4.34	4.77	1.07	1.20	4.56	1.13
X	4.36	4.40	1.07	1.12	4.38	1.10
XI	4.93	4.96	2.22	2.40	4.94	2.31
XII	4.75	4.59	2.13	2.51	4.67	2.32
XIII	4.52	4.34	2.19	2.64	4.43	2.42
XIV	4.00	4.04	2.52	2.72	4.02	2.62
XV	4.46	4.41	3.16	3.39	4.44	3.28
XVI	3.91	3.61	2.74	3.24	3.76	2.98
XVII	3.33	3.02	2.16	2.05	3.17	2.11
XVIII	3.31	3.31	1.13	1.09	3.31	1.11
XIX	2.98	2.91	1.32	1.29	2.94	1.30
XX	2.75	2.54	1.24	1.21	2.64	1.23
XXI	2.47 (6*)	2.16 (4*)	1.10 (6*)	1.15 (4*)	2.35 (10*)	1.12 (10*)
XXI′	2.60 (4*)	2.47 (6*)	1.66 (4*)	1.79 (6*)	2.52 (10*)	1.74 (10*)

* Number of chromosomes measured when less than 20.

observed with respect to % TCL. Females tend to have larger % TCL for long chromosomes, males tend to have larger % TCL for short chromosomes.

Although inconsistent treatment of centromeres could produce systematic effects of this kind, a more probable explanation is provided by the differential contraction postulated above to account for the reduced variation in length between homologous chromosomes of one nucleus. Indeed, when the mean absolute complement lengths of the two sexes are compared (data not shown) that of the female is found to be longer by a factor of 1.32. Since complements of greater absolute length have relatively larger long chromosomes it would appear that there must be a smaller degree of mitotic contraction for long chromosomes or chromosome arms.

Description of chromosome complement (idiogram)

The idiogram (Fig. 3) is constructed on the basis of the mean lengths and arm ratios shown in the last two columns of Table 3.

Major chromosome groups are placed in one row, the centromeres are aligned horizontally, the short arms face up, and chromosomes are

numbered by Roman numerals placed at the centromere. The mean haploid % TCL is indicated in Arabic numerals beneath each chromosome and the mean arm ratio similarly above.

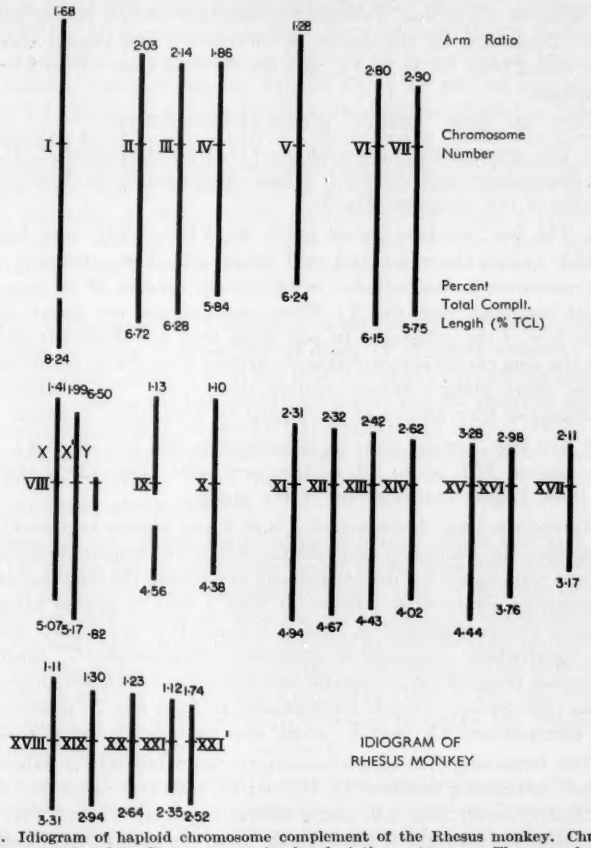


Fig. 3. Idiogram of haploid chromosome complement of the Rhesus monkey. Chromosomes are numbered by Roman numerals placed at the centromere. The mean haploid % total complement length (TCL) is indicated in Arabic numerals beneath each chromosome and the mean arm ratio similarly above

Within horizontal rows, individually distinguishable chromosomes or groups of chromosomes are set off from each other by double spacing. Within such groups, chromosomes that are not readily distinguishable are single spaced; the sex determining chromosomes, which are all individually distinct are shown in a compact group.

The heteromorphic alternates to the X chromosome (X′) and to chromosome XXI (XXI′) are also shown in the idiogram.

All autosomes of the Rhesus monkey are metacentric and all have arm ratios of 3.3 or less. In regard to size they form a closely graded series. Depending on the degree of contraction, the longest chromosome varied from 8.1 to 14.9 μ, and the shortest from 1.9 to 3.9 μ at metaphase.

There are three "natural" groups of chromosomes.

1. The seven "long" pairs No. I—VII, with the haploid % TCL of each chromosome well above 5.4. These chromosomes are shown in the top row of the idiogram (Fig. 3).

2. The ten "medium"-sized pairs, No. VIII—XVII, with haploid % TCL values above 3.1 and well below 5.4. The extremely short Y chromosome is also included in this group because of its presumed partial homology with the X. These chromosomes are shown in the center row of the idiogram. In size range they are absolutely distinct from the long chromosomes, but some overlap occurs with chromosomes of the short group. Where overlap does occur, the medium-sized chromosomes may always be recognized by arm ratios in excess of 2.

3. The four "small" pairs of chromosomes, No. XVIII—XXI, with a % haploid TCL up to 3.4, and an arm ratio approaching unity in the three longest chromosomes of the group.

Among the long chromosomes, I and V can always be individually identified. No. I is clearly and without overlap the longest chromosome, the arm ratio near 1.7 is distinctive, and in addition the long arm carries a secondary constriction (arrows in Figs. 1 and 2; gap in idiogram) which is always expressed at least as a kink. It is not known whether this constriction organizes a nucleolus. Chromosome V, fourth in decreasing order of size, is conspicuous through the submedian position of its centromere. Length distinguishes it from the X chromosome, and chromosomes IX, and X, which also have nearly equal arms.

The remaining five long chromosome pairs fall into 2 subgroups. A first, comprising numbers II, III and IV with arm ratios well below 2.4, and typically near 2.0, and a second (numbers VI and VII) with arm ratios well above 2.4, and typically near 3.0. In the first subgroup, II is clearly second in total length and has an arm ratio of almost exactly 2, III has an arm ratio distinctly above 2, and IV an arm ratio distinctly below 2. III and IV also differ appreciably in length, IV being really the 6th chromosome in a precisely graded series. Chromosomes VI and VII also can usually be differentiated from each other on the basis of total length. In clear preparations, all seven long chromosome pairs may be identified unequivocally.

The medium-sized chromosomes again fall into two subgroups, a first characterized by an arm ratio below 1.5 (except for X′ and Y) comprising the "longer" chromosomes VIII, IX and X; and a second characterized by arm ratios well above 2, and including the "shorter" chromosomes XI—XVII. All members of the first group are individually distinctive. The standard X chromosome, as found in all males and all but one of the females, has a % haploid TCL of about 5.1 and an arm ratio of about 1.4. Nuclei in a single female (two analyses included in Table 3), consistently had an unequal pair of X chromosomes (X, X′), the members of which did not differ clearly in overall length but differed unmistakably in arm ratio, that of X′ being 2.0. A structural rearrangement appears to be indicated. If there is really no difference in length, a pericentric inversion or centromere shift might be inferred. Any such specific hypothesis must however await corroboration from meiotic bivalent analysis.

The Y chromosome of the male could readily and consistently be identified in slides representing at least 12 different animals. It is extremely short, equivalent to less than 1% of the haploid complement. This is also true for the baboon *(Papio papio)* and the pigtailed monkey *(Macacus nemestrina)* studied by DARLINGTON and HAQUE (1955). The arm ratio is linearly larger than 5.0 and much larger volume-wise. In the nucleus shown in Fig. 1, the short arm is excessively stretched; it is, however, always distinct.

Pairs IX and X are almost alike in overall length and arm ratio but IX stands out by reason of the enormous secondary constriction (gap in idiogram) in the statistically longer arm. This constriction is almost certainly associated with the main nucleolus, but attempts to stain the body of the nucleolus in prophase have so far been unsuccessful. The main nucleolus in man also attaches medially according to SCHULTZ and ST. LAWRENCE (1949), and YERGANIAN (1957). The two arms of chromosome X which are virtually identical in length can not be distinguished by any structural landmark.

An arm ratio larger than 2 identifies chromosomes in the range % haploid TCL 2.8—5.4 as members of the second subgroup. Among these chromosomes, XVII with an arm ratio 2.1 is the only one individually identifiable with certainty. The remaining six pairs of chromosomes are best considered in two sections. First, of chromosomes XI to XIV which have arm ratios smaller than 2.8, XIV is the smallest and has distinctly the largest arm ratio, and can usually be recognized. The six chromosomes comprising pairs XI to XIII have been paired in all analysed nuclei, but it is not certain that such pairing was always genetically correct. Second, chromosomes XV and XVI always have

arm ratios well above 2.8, and in clear preparations can reliably be distinguished from each other by differences in length.

Among the four short pairs, XVIII and XIX can be distinguished from XX and XXI on the basis of overall length, and from each other on the basis of arm ratio. Chromosomes XX and XXI can not always be clearly identified.

The smallest chromosome has been found in two forms, XXI and XXI′, again apparently identical in overall size, but differing widely in arm ratios. The discussion given for the dimorphic X chromosomes is also pertinent here. Of the 10 nuclei analyzed, 4 (2 ♂, 2 ♀) were homozygous XXI, 4 (1 ♂, 3 ♀) were homozygous XXI′, and one male was heterozygous. It is not known how many animals were represented by these ten nuclei.

Summary

The chromosome complement of male and female Rhesus monkey has been investigated in kidney cells cultivated *in vitro* for 3 to 6 days. The chromosome number is 42. The Y chromosome of the heterogametic male is the smallest element in the complement, and it is acrocentric. The X chromosome ranks eigth in decreasing order of size and typically has an arm ratio of 1.4. The autosomes form a graded size series of metacentric chromosomes, 3—15 μ long in early metaphase, and with arm ratios from 1.1 to 3.3. Chromosome IX carries a large secondary constriction near the centromere; it is presumed to be the main nucleolar chromosome. A smaller secondary constriction is found consistently in the long arm of chromosome I. The X chromosome and chromosome XXI appear to be dimorphic in the limited population studied, the alternative forms differing in arm ratios but not in total length. An idiogram of the haploid chromosome complement is presented incorporating measurements of 10 completely analyzed nuclei, five from male monkeys and five from females. On the basis of relative length, arm ratio, and occurrence of secondary constrictions, most chromosomes of the complement can be individually identified.

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THE CONTROL OF CHIASMA FREQUENCY IN VICIA FABA L.

By
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With 1 Figure in the Text

(Eingegangen am 18. Dezember 1957)

The main immediate source of variability in a population, so necessary for its continued evolution, is genetic recombination. The amount however which occurs in each generation is closely correlated with the breeding system of the species, a high level promoting flexibility, which is required in a cross-fertilizing species, and a low level being characteristic of inbreeding plants. Recombination is under close genetic control so that the efficiency of the population is not unduly affected.

Since genetic recombination must be the outcome of crossing-over, its control occurs at the chromosome level (Rees 1953, 1955), but the actual mechanism has not yet been adequately described.

Since Morgan *et al.* (1933) demonstrated genetically that prevention of crossing-over on two of the long chromosomes of *Drosophila melanogaster* was accompanied by an increase in crossing-over in the third chromosome, several investigators have attempted to show the same relationship cytologically. Darlington (1933) found a negative correlation between the chiasma number of the small extra bivalent and the total number of chiasma of the seven large bivalents in eight-chromosome rye plants. Sax (1935) disputed this finding on examining chiasma frequency in *V. faba*, where he found that the chiasma frequency of the large *M* bivalents was uncorrelated with the total chiasma frequency of the five small *m* bivalents. Mather and Lamm (1935) confirmed the findings of both Darlington and Sax and showed that normal seven-chromosome rye plants exhibited a negative intra-class correlation, and similarly within the five small bivalents in *V. faba*. Mather (1936) examined data on chiasma frequency in 27 different organisms and found positive correlations of chiasma frequencies in some cases, and negative ones in others, while many organisms gave no significant correlations at all. Correlations which were statistically significant appeared in some organisms within groups of bivalents, and in others between groups. On the basis of these observations, Mather proposed his "Hypothesis of Competition" which suggested that the bivalents within a nucleus must compete for a limited number of chiasmata.

In an organism like *V. faba* where the chromosomes can be separated into two groups, both the correlation coefficient and comparison of inter- and intra-cell variances can be used to provide information about the existence of correlations in the material. Furthermore chiasma formations are more numerous than in many plants and terminalisation is almost non-existent (MAEDA 1930), thus allowing metaphase chiasma frequencies to be used with comparative safety.

Materials and methods

The plants used for study were representative types taken from a *Vicia* collection which had been built up at Aberystwyth over a number of years, and divided into the two main groups, *major* and *minor* respectively.

Separate flower buds were fixed in Carnoy (6:3:1) to which a few drops of saturated aqueous ferric chloride had been added. Fixations were made simultaneously and at comparative growth stages in an attempt to reduce to a minimum differences due to environment. Chiasma frequency examinations were made during metaphase in aceto-carmine squash preparations and correlation coefficients were calculated using the standard formula: $r = \frac{S(xy)}{S(x)^2 S(y)^2}$. From the analysis of variance, intra-class correlations could be determined. In the latter calculations, the individual small bivalents could not be recognized, so that the mean square within nuclei was used instead of the inherent mean square. MATHER (1936) has shown that unless great differences in size occur between the bivalents, the use of the mean square within nuclei is just as efficient in detecting intra-class correlations.

The minimum number of cells on which observations were to be made in order to give differences which are statistically significant was first determined. Two samples of twenty cells were taken from each of three individual plants, together with one of similar size from each of two unrelated plants. The distribution of chiasmata observed for each sample is shown in Table 1.

Table 1. *Chiasma frequency per cell in samples of 20 cells taken from the same and unrelated plants*

Variety	Chiasma frequency per cell									Mean per cell	St. dev. ±	„t"	Significance
	16	17	18	19	20	21	22	23	24				
FM 3/1	—	—	3	3	9	4	1	—	—	19.85	0.2433	2.93	**
FM 3/2	—	—	—	3	5	5	3	3	1		0.3282		
FM 18/2 A	1	2	3	7	5	2	—	—	—	18.95	0.2458	0.27	not sig.
B	1	2	3	9	3	2	—	—	—	18.85	0.2835		
FM 18/3 A	1	5	4	4	4	2	—	—	—	18.55	0.3282	0.12	not sig.
B	1	2	7	6	2	2	—	—	—	18.60	0.2846		
FM 2/5 A	1	3	2	5	5	2	2	—	—	19.20	0.3741	1.78	not sig.
B	1	—	2	3	6	3	4	1	—	20.15	0.3787		
Total	6	14	24	40	39	22	10	4	1	19.40	0.1280	—	—

** = significant at the 1% level

It can be seen that the samples from the unrelated plants showed highly significant differences, while both samples taken from the same plant did not show such differences. Twenty cells for each analysis were therefore considered to be a fair sample.

Results

A. Differences between V. faba major and V. faba minor

Since no information was available regarding any possible differences in chiasma frequency between *major* and *minor*, plants from each group were analysed separately. The results indicate differences between means within each group, while the groups themselves do not differ significantly from each other, "*t*" for 6 degrees of freedom being 0.003 giving a probability of over 90 per cent (Table 2).

Table 2. *Chiasma frequency in V. faba major and V. faba minor*

V. faba	Variety	Mean per cell	St. dev. ±	Group mean	St. dev. $\bar{x}$
Minor	Fm 3	20.60	0.2000	18.87	0.5967
	Fm 7	18.33	0.2274		
	Fm 2	17.90	0.3471		
	Fm 6	18.65	0.3787		
Major	FM 6	19.09	0.1179	18.89	0.2420
	FM 1	18.70	0.2683		
	FM 9	18.33	0.3971		
	FM 15	19.45	0.2755		

One can conclude therefore that the two groups do not differ in respect of chiasma frequency, which is in agreement with SENJANINOVA-KORCZAGINA (1932) who showed that the two groups are indistinguishable in chromosome size and in the relative length of chromosome segments in particular of the *M* chromosome.

B. Differences between plants and within plants

Since varieties as such cannot be recognized in *V. faba*, comparisons must be carried out on a plant basis, and although populations can be separated by analysis, chiasma frequency per plant appears to vary considerably within as well as between particular populations.

Table 3 gives the analysis of chiasma frequency in 20 cells for each of 16 plants.

Total sums of squares and inter-plant sums of squares can be calculated from the data, and intra-plant sums of squares obtained by difference. Calculation of the variances and a variance-ratio test of inter- and intra-plant mean squares shows (Table 4) that the inter-plant mean square is significantly greater than the intra-plant mean square.

These results suggest that whereas comparatively little variation occurs within each plant individual plants differ from each other appreciably in their level of chiasma frequency. Thus, it may be assumed that within each plant there is a tendency to control the total chiasma

Table 3. *Frequency of cells with stated number of chiasmata in V. faba*

Plant	Chiasmata per cell												
	12	13	14	15	16	17	18	19	20	21	22	23	24
FM 2	—	—	—	1	3	4	5	4	2	1	—	—	—
FM 6	—	—	—	—	1	4	7	2	3	1	2	—	—
FM 9	—	—	—	1	2	3	6	4	3	1	—	—	—
FM 15	—	—	—	—	—	—	5	7	3	4	1	—	—
FM 3/1	—	—	—	—	—	—	3	3	9	4	1	—	—
FM 18/2	—	—	—	—	1	2	3	7	5	2	—	—	—
FM 18/3	—	—	—	—	1	5	4	4	4	2	—	—	—
FM 18/4	1	—	2	1	6	4	4	1	—	1	—	—	—
FM 18/6	—	—	—	—	—	1	4	6	5	4	—	—	—
FM 18/8	—	—	—	—	1	2	6	7	3	—	1	—	—
FM 2/5	—	—	—	—	1	3	2	5	5	2	2	—	—
FM 2/6	—	—	—	—	1	1	4	5	3	3	2	1	—
J 0	—	—	—	—	—	—	2	6	5	5	1	1	—
J 2	—	—	—	—	—	1	4	1	4	3	4	1	2
J 4	—	—	—	—	2	5	3	4	—	3	—	1	2
J 8	—	—	—	—	—	1	—	9	3	3	2	1	1
Total	1	—	2	3	19	36	62	75	57	39	16	5	5

Table 4

	Sum of squares	N	Mean square	Mean square ratio	Probability
Inter-plants	284.3875	15	18.959	7.95	<0.001
Intra-plants	725.3000	304	2.386		
Total	1009.6875	319	—	—	—

frequency in each cell, but the level at which this control operates can differ from plant to plant.

C. Differences between and within cells of the same plant

Chiasma frequency of individual bivalents was noted in four separate plants although the particular bivalent involved each time could not itself be identified except the large *M* bivalent. The frequencies recorded singly for the small bivalents and their totals for each cell are shown in Table 5.

Table 5. *Frequency distribution of bivalents and cells for numbers of chiasmata*

Plant Ref. No.	Chiasmata per *m* bivalent				Total bivalents	Total chiasmata *m* bivalents								Total cells
	1	2	3	4		11	12	13	14	15	16	17	18	
J 0	—	35	53	12	100	—	3	4	7	5	1	—	—	20
J 2	—	43	45	12	100	2	3	4	7	3	1	—	—	20
J 4	2	41	44	13	100	3	5	4	3	2	1	1	1	20
J 8	—	32	55	13	100	—	3	6	4	3	2	2	—	20
Total	2	151	197	50	100	5	14	18	21	13	5	3	1	80

In the four plants analysed the range was small being from one to four chiasmata per bivalent. An analysis of these figures for the small bivalents of *V. faba* using the method of intra- and inter-cell variance is given in Table 6, and, for comparison, the figures obtained by MATHER

Table 6. *Comparison of intra- and inter-cell variance as an indication of correlation of chiasma frequency*

Plant Ref. No.	Variance		Variance ratio	Z	Probability
	intra-cell	inter-cell			
J 0	0.4600	0.2584	1.8	0.2939	0.05 (5%)
J 2	0.4800	0.3680	1.3	0.1312	0.05
J 4	0.4600	0.7870	1.71	0.2682	0.05
J 8	0.4000	0.4940	1.24	0.1076	0.05
MATHER and LAMM	0.7080	0.3531	—	0.3478	0.01

and LAMM (1935). Variance ratios were originally used, but for comparison with the data of MATHER and LAMM, they were transformed into values of Z.

Intra-cell variance was not always significantly greater than inter-cell variance, and in one plant it was much smaller, but in plant J 0, it was significantly greater suggesting a tendency to a negative correlation within the small bivalents as shown by MATHER (1936). Plant J 4, however, was the opposite, consequently it must be concluded that there is a tendency towards a positive correlation within the small bivalents. In the two plants J 2 and J 8, however, no significant differences were observed between their intra- and inter-cell mean squares.

Table 7. *Inter-class correlation coefficients for V. faba*

Plant Ref. No.	N	r
FM 3/1	18	−0.6201 **
FM 3/2	18	−0.5801 **
J 8	18	−0.2452
J 0	18	−0.2457
FM 2/5	18	−0.0217
FM 18/4	18	+0.2257
J 2	18	+0.2687
J 4	18	+0.3010

** significant at 1% level.

Calculation of correlation coefficients between the chiasma frequency of the large M bivalent and total chiasma frequency of the small bivalents per cell, shows a similar distribution of correlations, although not in the same plants. The data for eight selected plants are given in Table 7 in order of size from negative to positive.

Of these eight plants analysed, two gave significant negative correlations, but in the four plants previously analysed by the Z method, not one significant correlation was found. In effect, a wide range of coefficients can be seen, from negative correlations that are highly significant to positive which approach the level of significance. MATHER

(1936) showed that a significant deviation of the mean of the correlation coefficients from zero affords good evidence of a tendency to demonstrate correlations in the direction of that deviation, although it is better to test the corresponding Z values than the coefficients themselves. Testing the present range of coefficients in this way, with $t = \frac{0.0925}{0.2933}$, or 0.3153, and with N, the number of the degrees of freedom as 7, the probability is 0.8 (80 per cent). In fact, the deviation from zero is very slight indeed, and is not significant, which suggests a purely random distribution of the coefficients around zero. A similar range of correlation coefficients has been demonstrated recently by HARTE (1956) for the chromosomes of *Paeonia tenuifolia*, which suggests that this condition may be of more common occurrence than has been previously realised.

The disadvantage in analysing cells from a single plant, however large the sample, thus becomes apparent since by chance the particular plant selected may show a significant positive correlation, while the majority of plants would show no correlation at all.

D. Chiasma formation per bivalent

An analysis of chiasma frequency per bivalent in relation to interference is useful in deciding whether the particular plants analysed

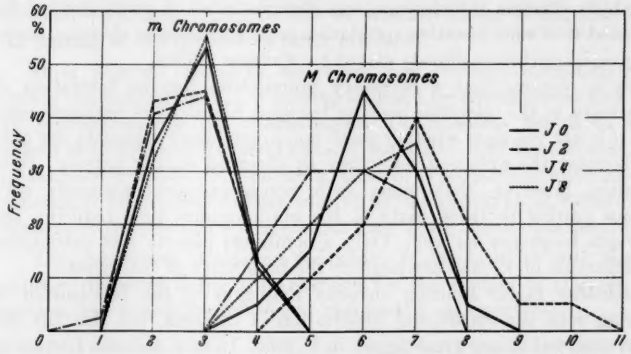

Fig. 1. Frequency of chiasmata per bivalent as an indication of chiasma interference

were abnormal in any respect. Data for m and the corresponding M bivalents in the four plants J0, J2, J4 and J8 are shown graphically in Fig. 1. According to HALDANE (1931) the presence of interference can be inferred when the frequency of chiasmata is too great near the mode and too little near the extremes on the expectation of

randomness. This test for departure from a Poisson distribution as an indication of interference gives the values in Table 8, where in all cases the variance is much less than the mean.

Table 8. *Comparison of mean and variance of chiasma frequency per bivalent in four plants of V. faba*

Plant Ref. No.	*m* chromosomes		*M* chromosomes	
	mean	variance	mean	variance
J 0	2.77	0.2548	6.15	0.8275
J 2	2.69	0.3680	7.05	1.4475
J 4	2.68	0.7870	5.65	1.0275
J 8	2.81	0.4940	6.05	1.1475
	Mean 2.74		Mean 6.23	

It can be seen that such is the case in both the *m* and *M* chromosomes, a distinct similarity can be detected between the present material and the data used by HALDANE (1931). The four plants under discussion must therefore be regarded as normal in so far as they show interference comparable with that demonstrated by previous workers.

Discussion

The results obtained show clearly that there is a tendency to control chiasma frequency within fairly close limits, but that variation can occur from plant to plant in the actual level which this control determines. It does appear, however, that the negative correlation of chiasma frequency is not in any way responsible for this control. Since a significant negative correlation was found only in one of the four plants in which chiasma interference was demonstrated it must further be assumed that such negative correlation is independent of chiasma interference, a conclusion already stated by MATHER (1936).

It is obvious that a necessary prerequisite for the formation of chiasmata is the adequate pairing between homologous chromosomes, but it is not yet clear whether small non-homologous segments are able to decrease the apparent homology as observed during pairing. It is possible, however, that many such non-homologous segments may reduce pairing in these parts of the chromosomes thus reducing the chiasma frequency as well. Only a combined genetic and cytological examination of *V. faba* can confirm the possibility of this effect.

Another factor limiting chiasma frequency is the localization of pairing with the consequent localization of chiasma, but this has not been observed to any great degree in *V. faba*. In fact, chiasma frequency appears to be largely a function of chromosome length, and when the bivalent number is constant, the control of chiasma frequency per cell would tend to operate within fairly narrow limits. Within each cell, however, variation in particular bivalents would rarely be in the same direction, the results being stabilization of chiasma frequency from cell to cell. To demonstrate a negative correlation of chiasma frequency in a number of cells in the same plant would require some factor common to

all the cells and controlling the chiasma frequency of particular bivalents in different directions. Such a factor could be chromosome pairing at pachytene. With long chromosomes, or on the other hand, a large number of chromosomes, mechanical interference between chromosomes could often reduce pairing especially if large size differences existed. It is only if chromosome reproduction takes place before pairing has been completed that the effects of interference will be apparent. Normally, although pairing sometimes may be slightly delayed, sufficient time may elapse before reproduction for pairing eventually to become complete. Consequently negative correlation would occur only when reproduction of the chromosomes was precipitated possibly by external conditions, and since meiosis within a particular anther is normally simultaneous in all the cells, the analysis of chiasma frequency based on a single anther under such conditions would show a highly significant negative correlation. The chance of this correlation appearing would be greater in plants with long chromosomes (generally with a high chiasma frequency) or with large numbers of chromosomes per cell, as observed by MATHER (1936).

MATHER's "Hypothesis of Competition" pre-supposes a limitation of some material within the nucleus for which the bivalents must compete. In one respect this is in accord with the idea of a time limit for pairing imposed by the onset of chromosome reproduction, since some bivalents by their very nature may be able to take advantage of the time limit for pairing at the expense of other bivalents.

It is possible, therefore, to re-establish chiasma formation on a purely mechanical basis, i. e. pairing followed by crossing-over and then by reproduction of the chromosomes. Chromosome length to some extent is under genetic control and also spiralization of the chromosomes (DARLINGTON 1932). Furthermore, time of reproduction must also be genetically controlled, so that control by the genotype of variation in chiasma frequency can operate on at least three levels, possibly more.

The correlation of chiasma frequency in one bivalent with that in another therefore is not a mechanism for the control of chiasma formation, but rather the infrequent outcome of such control.

Summary

In *Vicia faba* two groups of chromosomes can be distinguished and consequently correlations of chiasma frequency can be calculated between the groups and also within the group of small chromosomes. A negative correlation was demonstrated during some analyses while positive ones occurred in others, in fact a wide range of coefficients could be calculated by both methods.

It is suggested that negative correlation of chiasma frequency occurs only when reproduction of the chromosomes is precipitated or pairing delayed, so that the degree of pairing may be affected in plants where many chromosomes are present or where the chromosomes are large in size. Such correlations can have no influence on recombination, but rather they are the occasional outcome of the mechanism controlling chiasma frequency.

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DIFFERENTIAL BEHAVIOUR OF CHROMOSOMES IN SCILLA

By

H. REES

With 3 Figures in the Text

(Eingegangen am 28. November 1957)

1. Introduction

It is characteristic of the nuclei of most species that the chromosomes of which they are comprised contract or "condense" at an equal rate during prophase of cell divisions. In species where differences in condensation are normal occurrences they are associated with heterochromatic chromosomes or segments of chromosomes. Recently abnormal differences in condensation have been described by JAIN (1957) at meiosis in certain *Lolium* plants following heat treatment. His results have an important bearing on the progress of meiosis generally as well as on the nature of differential behaviour exhibited by chromosomes contained in the same cell. It is worthwhile giving a summary of JAIN's results before dealing with the present work which concerns related phenomena in a *Scilla* species. His results, briefly, were as follows:

1. In each of many pollen mother cells, at diplotene and later, two classes of chromosomes were distinguishable in respect of their degree of contraction. One class consisted of strongly contracted chromosomes, invariably paired by chiasmata. The second class was, relatively, retarded in that the chromosomes were less contracted. Frequently these were unpaired.

2. Nucleoli in the abnormal cells varied in number from one to six, as compared with one, sometimes two, in normal cells. The nucleoli were associated with contracted, never with the retarded chromosomes.

The following observations of abnormal meiosis in *Scilla* pollen mother cells make some further contributions towards the understanding of differential behaviour within cells. They also throw light on the specificity of response of particular chromosomes. This is possible in *Scilla* because unlike *Lolium*, the chromosomes to some degree can be distinguished by their lengths.

2. Material

The plants belonged to a diploid clone ($2n = 12$) of *Scilla sibirica*, variety Spring Beauty. The abnormal meiosis was discovered in two bulbs kept indoors. A bulb grown outside had normal meiosis, so that it is probable, especially in view of JAIN's work, that these "spontaneous" abnormalities were influenced by temperature.

Buds were fixed in acetic alcohol, stained in aceto-carmine.

3. General description

In one way or another meiosis was irregular in almost all pollen mother cells in the indoor bulbs. With rare exceptions the chiasma frequency was extremely low, the average about 3.0 per cell, and less than one per cent of the cells contained no univalents. Differential condensation was manifested by more than 50 per cent of cells. Associated with these main effects were poor congression and orientation at metaphase, abnormal, frequently split, spindles at anaphase (Fig. 2a), and in rare cells extensive chromosome breakage (Fig. 3e). Since the asynaptic cells contained either equally or differentially condensed chromosomes the two effects must be independent, although probably they arise from the same cause. Defective metaphase arrangements of chromosomes as well as the abnormality of spindles are commonly associated with asynapsis (see Darlington 1937) although to some extent, as Jain found, the irregular orientation of some bivalents may well reflect uncoordination between the centromeres in respect of their orientation and the development of the spindle, a timing anomaly to be expected where chromosomes within the same cell are not synchronised in their division cycles.

4. Cells with differentially condensed chromosomes

Within these pollen mother cells from diplotene to metaphase the chromosomes fall into two or three classes showing different degrees of stainability and contraction (see Fig. 3). The occurrence of three classes is in contrast to Jain's *Lolium* results, where two only were distinguished in any one cell. It may be that the greater variation of response in *Scilla* indicates greater genetic diversity between members of the chromosome complement. This is not an unreasonable assumption when one compares the chromosomes of the two species on the basis of length: in *Lolium* there is comparitive uniformity, in *Scilla* considerable variety.

The extent to which chromosomes differ within the cell (see Fig. 3) is variable, as in *Lolium*. There is in *Scilla* however no correlation between the degree of retardation and the number of advanced chromosomes as there is in *Lolium*. This was shown by scoring the number of advanced chromosomes in a sample of cells exhibiting extreme retardation. The results are represented in the graph (Fig. 1), from which it will be seen that extreme differences between chromosomes are found in cells with few or with many advanced chromosomes. The graph is instructive in another way viz. that odd numbers of advanced chromosomes are found in the same cell, although more frequently, as the peaks of the graph indicate, the numbers are even. It is clear from these data, and from general observation of cells (e.g. Fig. 3a) that homologous chromo-

tomes do not always respond in the same way, although more commonly they do. The difference from *Lolium* in this respect is worth considering. There, as pointed out earlier, advanced chromosomes are always present in even numbers. It will be remembered too that the homologues in *Lolium* not only behave alike but when condensed always have formed chiasmata. It is tempting to attribute the similarity of their behaviour to their proximity consequent upon chiasma formation, and the diverse behaviour of some homologues in *Scilla* to their separateness consequent upon asynapsis. Furthermore we must note in this connection that while univalents in *Scilla* may not always be synchronised, members of a bivalent always are. Some additional evidence relating to this possibility will be discussed later.

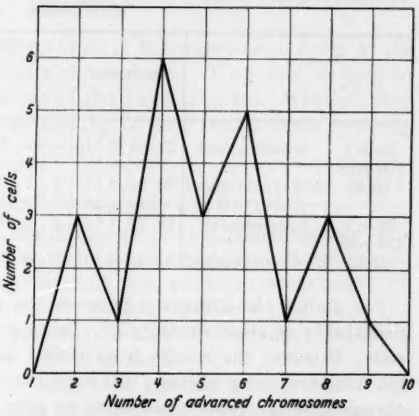

Fig. 1. Graph of the frequency distributions of advanced chromosomes in pollen mother cells exhibiting extreme differential behaviour

Specificity. It is unfortunate that the chromosomes could not be completely classified during meiosis in these cells. It was however possible to pick out the longer chromosomes from the shorter. To determine whether the condensation of the chromosomes was in any way related to size, and hence to particular chromosomes, cell samples were scored in both bulbs. Despite the confusion of the classification based on length by differences in contraction it was evident that the long chromosomes were more often retarded than short chromosomes.. Within 22 out of 23 cells that could confidently be analysed retardation was greatest in longer chromosomes. That there are exceptions one would indeed expect from the fact that even homologous chromosomes may behave differently. In general however the relation holds good.

Chiasmata. The short chromosomes, generally the more advanced as we have seen, rarely form chiasmata. Long chromosomes on the other hand frequently do so. In the 23 cells dealt with in the previous section the average chiasma frequency of the longest chromosome pair was 0.90, and in the shortest pair it was less than 0.05. We need to know whether the ability or inability of particular chromosomes to form chiasmata in these cells is related to their degree of condensation, or alternatively to

chromosome length independently of this. One way to find out is to compare the chiasma distributions of cells in which the chromosomes are synchronised with those in which they are not. If differential condensation affects the chiasma frequency we would expect the variances of chiasma frequencies between bivalents to be greater within unsynchronised cells than in the others. Comparisons were made between samples from each bulb. The data appear in Table 1.

Table 1. *The chiasma frequencies in pollen mother cells with and without differential condensation*

		Chiasma frequency												
		Bivalents				Cells								Variance between bivalents within cells
		0	1	2	3	0	1	2	3	4	5	6	7	
Bulb 1 (10 cells each)	Synchronised	28	26	6	0	—	—	3	0	5	1	0	1	0.45
	Not synchronised	36	21	3	0	1	1	3	1	3	1	—	—	0.35
Bulb 2 (15 cells each)	Synchronised	63	26	1	0	2	5	4	2	1	1	—	—	0.22
	Not synchronised	55	32	3	0	2	0	4	7	1	1	—	—	0.32

In Bulb 2 the difference between the variances within cells has a probability of approximately 5%, being greater in the unsynchronised cells. However the results from Bulb 1 are completely contradictory, the variance being greater, although not significantly so, in the synchronised cells. There is therefore no good evidence for a direct causal relation here between condensation and chiasma formation and since condensed bivalents are occasionally found with uncondensed univalents in the same cell it must be concluded that the chiasma frequency in the main at least is related to length *per se*.

Nucleoli. Normally in *Scilla sibirica* one or two nucleoli appear at diplotene. In contrast up to six or more nucleoli are found in these abnormal plants. The organisation of nucleolar material, as in *Lolium*, is disrupted.

Although in *Lolium* the nucleoli without exception were attached to advanced chromosomes, Jain rejected on various grounds the hypothesis that the attachment of nucleoli was directly related to chromosome condensation. His conclusion is borne out in *Scilla*, paradoxically, by a totally different association of nucleoli and chromosomes from that found in *Lolium*. Nucleoli in *Scilla* are associated with both advanced and retarded chromosomes. There is therefore no doubt that the attachment of nucleoli is not a prerequisite for condensation in these cells. One must however question whether the possibility of deficiency, actual or functional, of nucleolar material (ribo-nucleoproteins) causing failure of condensation can be entirely ruled out. One of Jain's main objections to this is based on the work of Jacobsen and Webb (1952) who showed

that ribo-nucleoproteins are deposited on the chromosomes at mitosis before the nucleolus diminished. It has since been shown however that the amount of RNA for example can vary in the nucleolus to some degree independently of its size (Lin 1955). It is therefore not impossible that retardation in *Lolium* and in *Scilla* may be due to a deficiency of, or failure to utilize, nucleolar material. There is no evidence that this is so, but it seems worthwhile pointing out that the evidence to the contrary is not entirely conclusive. At the same time the evidence from *Scilla* in no way contradicts Jain's view that DNA synthesis in the retarded chromosomes is abnormal.

As La Cour (1951) has shown blobs of heterochromatin occur at the ends of a number of the *Scilla* chromosomes. They can be seen to condense in advance of the rest of the arms (Fig. 3c). Whether they play any special part in the condensation of these chromosomes generally is not known.

5. Spatial relations of chromosomes and fragments

Two kinds of fragments found in some of the pollen mother cells provide an useful means of inquiry into some aspects of differential behaviour. The first, a long fragment, was produced occasionally by inversion crossing over in one of the long bivalents (Fig. 2b). The second, a smaller fragment, probably, though not certainly, was not a product of inversion crossing over (Fig. 2c, d).

The long fragment, in all cases held attached to the rest of the bivalent by chromatid attraction, was found to be synchronised with the chromosomes from which is was derived. Evidently whether centric or acentric did not directly affect the condensation-subsequent that is to crossing over when the fragment was cut off.

The short fragment in five out of the six cells in which it appeared, was, unlike the long fragment, detached and separate from its parent chromosome or chromosomes. In the one case it was held in proximity to a chromosome by a sticky matrix (Fig. 2c). What is significant is that whereas the detached fragments (Fig. 2d) were more advanced than any other chromosome in the nucleus and therefore not synchronised with their centric counterparts, the attached short fragment (Fig. 2c), like the attached longs, was synchronised with the chromosomes in its vicinity. It would appear that what determines whether the fragments, long or short, are synchronised with their complementary segments is their proximity or otherwise. There is therefore good reason to suppose that the chromosome segments co-operate, and are therefore synchronised, over limited distances, whereas they are unable to co-operate over longer distances and consequently are not synchronised. It will be recalled that there was some evidence suggesting that the homologous chromosomes sometimes behave differently in *Scilla* because of their asynapsis

and hence their unattachment. In view of the above observations this would be a most feasible conclusion. The conception of co-operation of closely associated chromatin within these nuclei is further reinforced by the synchronisation of chromosomes within groups that, despite squashing, can occasionally be clearly distinguished in some cells (Fig. 3b).

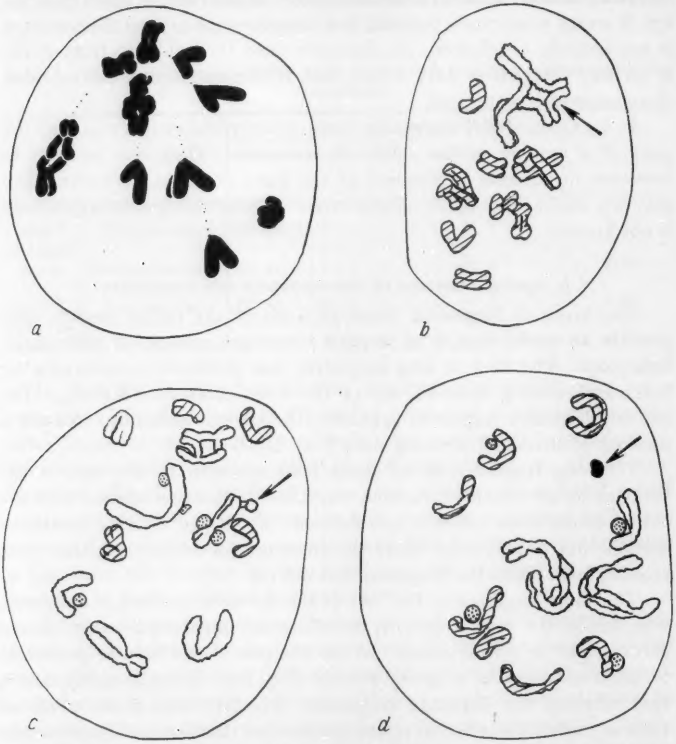

Fig. 2a—d. a Abnormal spindle at first anaphase. Chromosome movement is mainly towards one pole. b—c. Diakinesis. b A large fragment is attached to the long bivalent and in respect of condensation, synchronised with it. c A short fragment, attached by stickiness, is synchronised with the chromosome in its vicinity. Note the 5 nucleoli (dotted). d A short, detached, fragment not synchronised with the rest of the chromosome complement. Unshaded chromosomes are retarded, black chromosomes are advanced and the cross-hatched chromosomes intermediate. ×800

From a genetical standpoint another possible cause of the differentiation within the nuclei must be considered, that is variation in the immediate external environment of the chromosomes – the cytoplasm, or extrachromosomal material inside the nuclei. That the cytoplasm is

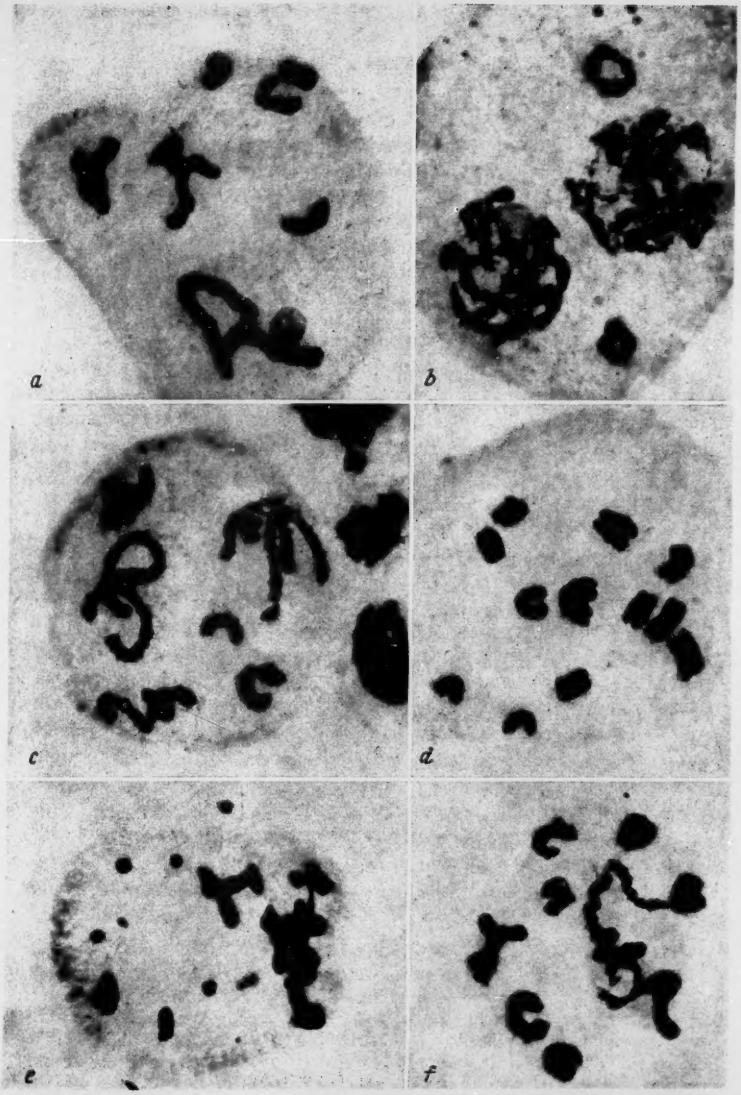

Fig. 3a—f. a Three advanced univalents, with two bivalents and five univalents slightly retarded. b Three stages of condensation in one cell. Note synchronisation within the two retarded sub-nuclei, one of which has a nucleolus. c Univalents and one long bivalent with a single chiasma are retarded. d Slight retardation in six of the twelve univalents. e Extreme fragmentation at metaphase. f One long bivalent retarded. ×950

concerned with the abnormality there is little doubt because in the same anthers we find genetically identical nuclei behaving differently in different cells. The differential behaviour of chromosome groups within the cells also, in so far as it is independent of their constituent chromosomes, must, on any deterministic grounds, be initiated by causes external to them. Such a view does not exclude that of co-operation between chromosomes in close association. Indeed the latter would be expected to reinforce and amplify the initial differences within the limits of the co-operating groups. The two causes in fact need not be regarded as alternatives but complementary to one another.

6. Conclusion

While the cause of the differential behaviour is not known we can with some confidence specify the conditions affecting its consequences. In the first place particular chromosomes respond to the abnormal conditions within the cell in different ways. In *Scilla* it was shown that long chromosomes were more frequently retarded than short ones. A specific chromosome response was also deduced by Jain in *Lolium*, from the disproportionate preponderance of unsynchronised cells with one or two advanced bivalents. This kind of specificity may or may not be simply related to length, for example the minimum requirements for condensation may be greater for longer chromosomes. There is no evidence either way.

A second condition, along with the first, would certainly appear to be the spatial relations of chromosome material within the nucleus, a factor recently discussed in general terms by Darlington (1957). On this basis the abnormal cells could be regarded as containing nuclei where co-operation was restricted, uncharacteristically, over limited distances. As suggested earlier the initial cause of this is most reasonably attributed to differences in the cytoplasm, which in normal cells permits general co-operation within nuclei, but which in abnormal cells with genetically identical nuclei, does not.

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